



INTERLEUKIN-3 (IL-3) MULTIPLE MUTATION POLYPEPTIDES

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This is a continuation-in-part of United States
Application Serial No. 07/981,044 filed November 24,
5 1992 which is incorporated herein by reference.

Field of the Invention

The present invention relates to mutants or
variants of human interleukin-3 (hIL-3) which contain
10 multiple amino acid substitutions and which may have
portions of the native hIL-3 molecule deleted. These
hIL-3 multiple mutation polypeptides retain one or
more activities of native hIL-3 and may also show
improved hematopoietic cell-stimulating activity
15 and/or an improved activity profile which may include
reduction of undesirable biological activities
associated with native hIL-3.

Background of the Invention

20 Colony stimulating factors (CSFs) which stimulate
the differentiation and/or proliferation of bone
marrow cells have generated much interest because of
their therapeutic potential for restoring depressed
levels of hematopoietic stem cell-derived cells. CSFs
25 in both human and murine systems have been identified
and distinguished according to their activities. For
example, granulocyte-CSF (G-CSF) and macrophage-CSF
(M-CSF) stimulate the in vitro formation of
neutrophilic granulocyte and macrophage colonies,
30 respectively while GM-CSF and interleukin-3 (IL-3)
have broader activities and stimulate the formation of
both macrophage, neutrophilic and eosinophilic
granulocyte colonies. IL-3 also stimulates the
formation of mast, megakaryocyte and pure and mixed
35 erythroid colonies.

Because of its ability to stimulate the

proliferation of a number of different cell types and to support the growth and proliferation of progenitor cells, IL-3 has potential for therapeutic use in restoring hematopoietic cells to normal amounts in those cases where the number of cells has been reduced due to diseases or to therapeutic treatments such as radiation and chemotherapy.

Interleukin-3 (IL-3) is a hematopoietic growth factor which has the property of being able to promote the survival, growth and differentiation of hematopoietic cells. Among the biological properties of IL-3 are the ability (a) to support the growth and differentiation of progenitor cells committed to all, or virtually all, blood cell lineages; (b) to interact with early multipotential stem cells; (c) to sustain the growth of pluripotent precursor cells; (d) to stimulate proliferation of chronic myelogenous leukemia (CML) cells; (e) to stimulate proliferation of mast cells, eosinophils and basophils; (f) to stimulate DNA synthesis by human acute myelogenous leukemia (AML) cells; (g) to prime cells for production of leukotrienes and histamines; (h) to induce leukocyte chemotaxis; and (i) to induce cell surface molecules needed for leukocyte adhesion.

Mature human interleukin-3 (hIL-3) consists of 133 amino acids. It has one disulfide bridge and two potential glycosylation sites (Yang, et al., CELL 47:3 (1986)).

Murine IL-3 (mIL-3) was first identified by Ihle, et al., J. IMMUNOL. 126:2184 (1981) as a factor which induced expression of a T cell associated enzyme, 20-hydroxysteroid dehydrogenase. The factor was purified to homogeneity and shown to regulate the growth and differentiation of numerous subclasses of early hematopoietic and lymphoid progenitor cells.

In 1984, cDNA clones coding for murine IL-3 were isolated (Fung, et al., NATURE 307:233 (1984) and Yokota, et al., PROC. NATL. ACAD. SCI. USA 81:1070 (1984)). The murine DNA sequence coded for a
5 polypeptide of 166 amino acids including a putative signal peptide.

The gibbon IL-3 sequence was obtained using a gibbon cDNA expression library. The gibbon IL-3 sequence was then used as a probe against a human
10 genomic library to obtain a human IL-3 sequence.

Gibbon and human genomic DNA homologues of the murine IL-3 sequence were disclosed by Yang, et al., CELL 47:3 (1986). The human sequence reported by Yang, et al. included a serine residue at position 8
15 of the mature protein sequence. Following this finding, others reported isolation of Pro⁸ hIL-3 cDNAs having proline at position 8 of the protein sequence. Thus it appears that there may be two allelic forms of hIL-3.

20 Dorssers, et al., GENE 55:115 (1987), found a clone from a human cDNA library which hybridized with mIL-3. This hybridization was the result of the high degree of homology between the 3' noncoding regions of mIL-3 and hIL-3. This cDNA coded for an hIL-3 (Pro⁸)
25 sequence.

U.S. 4,877,729 and U.S. 4,959,454 disclose human IL-3 and gibbon IL-3 cDNAs and the protein sequences for which they code. The hIL-3 disclosed has serine rather than proline at position 8 in the protein
30 sequence.

Clark-Lewis, et al., SCIENCE 231:134 (1986) performed a functional analysis of murine IL-3 analogues synthesized with an automated peptide synthesizer. The authors concluded that the stable
35 tertiary structure of the complete molecule was required for full activity. A study on the role of

the disulfide bridges showed that replacement of all four cysteines by alanine gave a molecule with 1/500th the activity as the native molecule. Replacement of two of the four Cys residues by Ala(Cys⁷⁹, Cys¹⁴⁰ -> Ala⁷⁹, Ala¹⁴⁰) resulted in an increased activity. The authors concluded that in murine IL-3 a single disulfide bridge is required between cysteines 17 and 80 to get biological activity that approximates physiological levels and that this structure probably stabilizes the tertiary structure of the protein to give a conformation that is optimal for function. (Clark-Lewis, et al., PROC. NATL. ACAD. SCI. USA 85:7897 (1988)).

International Patent Application (PCT) WO 88/00598 discloses gibbon- and human-like IL-3. The hIL-3 contains a Ser⁸ -> Pro⁸ replacement. Suggestions are made to replace Cys by Ser, thereby breaking the disulfide bridge, and to replace one or more amino acids at the glycosylation sites.

EP-A-0275598 (WO 88/04691) illustrates that Ala¹ can be deleted while retaining biological activity. Some mutant hIL-3 sequences are provided, e.g., two double mutants, Ala¹ -> Asp¹, Trp¹³ -> Arg¹³ (pGB/IL-302) and Ala¹ -> Asp¹, Met³ -> Thr³ (pGB/IL-304) and one triple mutant Ala¹ -> Asp¹, Leu⁹ -> Pro⁹, Trp¹³ -> Arg¹³ (pGB/IL-303).

WO 88/05469 describes how deglycosylation mutants can be obtained and suggests mutants of Arg⁵⁴Arg⁵⁵ and Arg¹⁰⁸Arg¹⁰⁹Lys¹¹⁰ might avoid proteolysis upon expression in Saccharomyces cerevisiae by KEX2 protease. No mutated proteins are disclosed. Glycosylation and the KEX2 protease activity are only important, in this context, upon expression in yeast.

WO 88/06161 mentions various mutants which theoretically may be conformationally and antigenically neutral. The only actually performed

mutations are Met² -> Ile² and Ile¹³¹ -> Leu¹³¹. It is not disclosed whether the contemplated neutralities were obtained for these two mutations.

WO 91/00350 discloses nonglycosylated hIL-3 analog proteins, for example, hIL-3 (Pro⁸Asp¹⁵Asp⁷⁰), Met³ rhuIL-3 (Pro⁸Asp¹⁵Asp⁷⁰); Thr⁴ rhuIL-3 (Pro⁸Asp¹⁵Asp⁷⁰) and Thr⁶ rhuIL-3 (Pro⁸Asp¹⁵Asp⁷⁰). It is said that these protein compositions do not exhibit certain adverse side effects associated with native hIL-3 such as urticaria resulting from infiltration of mast cells and lymphocytes into the dermis. The disclosed analog hIL-3 proteins may have N termini at Met³, Thr⁴, or Thr⁶.

WO 91/12874 discloses cysteine added variants (CAVs) of IL-3 which have at least one Cys residue substituted for a naturally occurring amino acid residue.

Summary of the Invention

The present invention relates to recombinant human interleukin-3 (hIL-3) variant or mutant proteins (muteins). These hIL-3 muteins contain amino acid substitutions and may also have amino acid deletions at either/or both the N- and C- termini. Preferably, these mutant polypeptides of the present invention contain four or more amino acids which differ from the amino acids found at the corresponding positions in the native hIL-3 polypeptide. The invention also relates to pharmaceutical compositions containing the hIL-3 muteins, DNA coding for the muteins, and methods for using the muteins. Additionally, the present invention relates to recombinant expression vectors comprising nucleotide sequences encoding the hIL-3 muteins, related microbial expression systems, and processes for making the hIL-3 muteins using the microbial expression systems.

The present invention includes mutants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been deleted from the C-terminus, and in which multiple amino acid substitutions have been made. Preferred muteins of the present invention are those in which amino acids 1 to 14 have been deleted from the N-terminus, amino acids 126 to 133 have been deleted from the C-terminus, and which also contain from about four to about twenty-six amino acid substitutions in the polypeptide sequence. These hIL-3 multiple mutation polypeptides may have biological activities similar to or better than hIL-3 and, in some cases, may also have an improved side effect profile, i.e., some muteins may have a better therapeutic index than native hIL-3. The present invention also provides muteins which may function as IL-3 antagonists or as discrete antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols. In addition to the use of the hIL-3 multiple mutation polypeptides of the present invention in vivo, it is envisioned that in vitro uses would include the ability to stimulate bone marrow and blood cell activation and growth before infusion into patients.

Antagonists of hIL-3 would be particularly useful in blocking the growth of certain cancer cells like AML, CML and certain types of B lymphoid cancers. Other conditions where antagonists would be useful include those in which certain blood cells are produced at abnormally high numbers or are being activated by endogenous ligands. Antagonists would effectively compete for ligands, presumably naturally occurring hemopoietins including and not limited to IL-3, GM-CSF and IL-5, which might trigger or augment the growth of cancer cells by virtue of their ability

to bind to the IL-3 receptor complex while intrinsic activation properties of the ligand are diminished. IL-3, GM-CSF and/or IL-5 also play a role in certain asthmatic responses. An antagonist of the IL-3
5 receptor may have the utility in this disease by blocking receptor-mediated activation and recruitment of inflammatory cells.

Brief Description of the Drawings

10 Figure 1 is the human IL-3 gene for E. coli expression (pMON5873), encoding the polypeptide sequence of natural (wild type) human IL-3 [SEQ ID NO:128], plus an initiator methionine, as expressed in E. coli, with the amino acids numbered from the N-
15 terminus of the natural hIL-3.

Figure 2: ClaI to NsiI Replacement Fragment.
Figure 2 shows the nucleotide sequence of the replacement fragment used between the ClaI and NsiI sites of the hIL-3 gene. The codon choice used in the
20 fragment corresponds to that found in highly expressed E. coli genes (Gouy and Gautier, 1982). Three new unique restriction sites, EcoRV, XhoI and PstI were introduced for the purpose of inserting synthetic gene fragments. The portion of the coding sequence shown
25 encodes hIL-3 amino acids 20-70.

Figure 3 shows the nucleotide and amino acid sequence of the gene in pMON5873 with the sequence extending from NcoI through HindIII. The codon choices used to encode amino acids 1-14 and 107-133
30 correspond to that found in highly expressed E. coli genes.

Figure 4 shows the construction of the plasmid vector pMON5846 which encodes [Met-(1-133) hIL-3 (Arg129)].

35 Figure 5 shows the construction of the plasmid vector pMON5847 (ATCC 68912) which encodes [Met-(1-

133) hIL-3 (Arg129)].

Figure 6 shows the construction of plasmid vector pMON5853 which encodes [Met-(15-133) hIL-3 (Arg129)].

Figure 7 shows the construction of the plasmid
5 vector pMON5854 which encodes [Met-(1-133) hIL-3 (Arg129)].

Figure 8 shows the DNA sequence and resulting amino acid sequence of the LamB signal peptide.

Figure 9 shows the construction of the plasmid
10 vector pMON5978 which encodes Met-Ala-(15-125)hIL-3.

Figure 10 shows the construction of the plasmid vector pMON5988 which encodes Met-Ala(15-125)hIL-3.

Figure 11 shows the construction of the plasmid vector pMON5887 which encodes Met-(1-125)hIL-3.

15 Figure 12 shows the construction of pMON6457 which encodes (15-125)hIL-3; it contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 13 shows the construction of pMON6458; it
20 contains the araBAD promoter and the LamB signal peptide fused to the variant hIL-3 amino acids 15-125.

Figure 14 shows the construction of pMON13359.

Figure 15 shows the construction of pMON13352.

Figure 16 shows the construction of pMON13360.

25 Figure 17 shows the construction of pMON13363.

Figure 18 shows the construction of pMON13364.

Figure 19 shows the construction of pMON13365.

Figure 20 shows the construction of pMON13287.

Figure 21 shows the construction of pMON13288.

30 Figure 22 shows the construction of pMON13289.

Figure 23 shows the construction of pMON5723.

Figure 24 shows the construction of pMON13438.

Detailed Description of the Invention

35 The present invention relates to muteins of human interleukin-3 (hIL-3) in which amino acid

substitutions have been made at four or more positions in amino acid sequence of the polypeptide and to muteins which have substantially the same structure and substantially the same biological activity.

5 Preferred muteins of the present invention are (15-125)hIL-3 deletion mutants which have deletions of amino acids 1 to 14 at the N-terminus and 126 to 133 at the C-terminus and which also have four or more amino acid substitutions in the polypeptide and
10 muteins having substantially the same structure and substantially the same biological activity. Among the preferred muteins are those having twenty-six amino acid substitutions. As used herein human interleukin-3 corresponds to the amino acid sequence (1-133) as
15 depicted in Figure 1 and (15-125) hIL-3 corresponds to the 15 to 125 amino acid sequence of the hIL-3 polypeptide. Naturally occurring variants of hIL-3 polypeptide amino acids are also included in the present invention (for example, the allele in which
20 proline rather than serine is at position 8 in the hIL-3 polypeptide sequence) as are variant hIL-3 molecules which are modified post-translationally (e.g. glycosylation).

The present invention also includes the DNA
25 sequences which code for the mutant polypeptides, DNA sequences which are substantially similar and perform substantially the same function, and DNA sequences which differ from the DNAs encoding the muteins of the invention only due to the degeneracy of the genetic
30 code.

Included in the present invention are novel mutant human interleukin-3 polypeptides comprising a polypeptide having the amino acid sequence of native human interleukin-3 wherein amino acids 126 to 133
35 have been deleted from the C-terminus of the native human interleukin-3 polypeptide and amino acids 1 to

14 have been deleted from the N-terminus of the native human interleukin-3 polypeptide and, in addition, polypeptides also have four or more amino acid substitutions in the polypeptide sequence.

5 Also included in the present invention are the DNA sequences coding for the muteins of the present invention; the oligonucleotide intermediates used to construct the mutant DNAs; and the polypeptides coded for by these oligonucleotides. These polypeptides may
10 be useful as antagonists or as antigenic fragments for the production of antibodies useful in immunoassay and immunotherapy protocols.

The mutant hIL-3 polypeptides of the present invention may also have methionine, alanine, or methionine-alanine residues inserted at the N-terminus.

The present invention includes human interleukin-3 mutant polypeptide Formula I:

20

Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

25 20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Xaa Xaa Xaa

35 40 45

[illegible]

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

65 70 75

35

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

11

80

85

90

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

95

100

105

5

Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

110

115

120

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID

10 NO:15]

125

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wherein Xaa at position 17 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

15 Xaa at position 18 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 19 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

Xaa at position 20 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 21 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn, Thr, Ser or Val;

20 Xaa at position 22 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln, Leu, Val or Gly;

Xaa at position 23 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe, Leu, Ser, or Arg;

Xaa at position 24 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

25 Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 26 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

Xaa at position 27 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 28 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 29 is Gln, Asn, Leu, Pro, Arg, or Val;

30 Xaa at position 30 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or Lys;

Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 32 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

35 Xaa at position 33 is Pro, Leu, Gln, Ala, Thr, or Glu;

Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr,

- Arg, Ala, Phe, Ile or Met;
- Xaa at position 35 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;
- Xaa at position 36 is Asp, Leu, or Val;
- Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
- 5 Xaa at position 38 is Asn, or Ala;
- Xaa at position 40 is Leu, Trp, or Arg;
- Xaa at position 41 is Asn, Cys, Arg, Leu, His, Met, or Pro;
- Xaa at position 42 is Gly, Asp, Ser, Cys, Asn, Lys, Thr, Leu, Val, Glu, Phe, Tyr, Ile, Met or Ala;
- 10 Xaa at position 43 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys, Gln, Arg, Thr, Gly or Ser;
- Xaa at position 44 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu, Asn, Gln, Ala or Pro;
- Xaa at position 45 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys, Trp, Asp, Asn, Arg, Ser, Ala, Ile, Glu or His;
- 15 Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln, Lys, His, Ala, Tyr, Ile, Val or Gly;
- Xaa at position 47 is Ile, Gly, Val, Ser, Arg, Pro, or His;
- Xaa at position 48 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu, Lys, Thr, Ala, Met, Val or Asn;
- 20 Xaa at position 49 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
- Xaa at position 50 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser, Ala, Ile, Val, His, Phe, Met or Gln;
- Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- 25 Xaa at position 52 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
- Xaa at position 53 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser, or Met;
- Xaa at position 54 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn, Lys, His, Ala or Leu;
- 30 Xaa at position 55 is Arg, Thr, Val, Ser, Leu, or Gly;
- Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His, Thr, Ala, Tyr, Phe, Leu, Val or Lys;
- Xaa at position 57 is Asn or Gly;
- Xaa at position 58 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
- 35 Xaa at position 59 is Glu Tyr, His, Leu, Pro, or Arg;
- Xaa at position 60 is Ala, Ser, Pro, Tyr, Asn, or Thr;

- Xaa at position 61 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
Xaa at position 62 is Asn His, Val, Arg, Pro, Thr, Asp, or Ile;
Xaa at position 63 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
- 5 Xaa at position 64 is Ala, Asn; Pro, Ser, or Lys;
Xaa at position 65 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 67 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
- 10 Xaa at position 68 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
Xaa at position 70 is Asn, Leu, Val, Trp, Pro, or Ala;
- 15 Xaa at position 71 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln, Trp, or Asn;
Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
- 20 Xaa at position 74 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 75 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser, Gln, or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
- 25 Xaa at position 77 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 78 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
Xaa at position 79 is Lys, Thr, Asn, Met, Arg, Ile, Gly, or Asp;
- 30 Xaa at position 80 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 81 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
- 35 Xaa at position 82 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn, His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
Xaa at position 83 is Pro, Ala, Thr, Trp, Arg, or Met;

- Xaa at position 84 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 85 is Leu, Asn, Val, or Gln;
Xaa at position 86 is Pro, Cys, Arg, Ala, or Lys;
Xaa at position 87 is Leu, Ser, Trp, or Gly;
5 Xaa at position 88 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 89 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn,
or Ser;
Xaa at position 90 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or
Met;
10 Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 92 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile
or Leu;
Xaa at position 93 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;
Xaa at position 94 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys,
15 His, Ala,
or Pro;
Xaa at position 95 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr,
Asn, Lys,
Ser, Ala, Trp, Phe, Ile, or Tyr;
20 Xaa at position 96 is Pro, Lys, Tyr, Gly, Ile, or Thr;
Xaa at position 97 is Ile, Val, Lys, Ala, or Asn;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr,
Glu, Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;
Xaa at position 99 is Ile, Leu, Arg, Asp, Val, Pro, Gln,
25 Gly, Ser, Phe, or His;
Xaa at position 100 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln,
or Pro;
Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,
Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu, or Gln;
30 Xaa at position 102 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;
Xaa at position 103 is Asp, or Ser;
Xaa at position 104 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu,
Gln, Lys, Ala, Phe, or Gly;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
35 Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;

15

Xaa at position 108 is Arg, Lys, Asp, Leu, Thr, Ile, Gln, His,
Ser, Ala

or Pro;

Xaa at position 109 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;

5 Xaa at position 110 is Lys, Ala, Asn, Thr, Leu, Arg, Gln, His,
Glu, Ser,

Ala, or Trp;

Xaa at position 111 is Leu, Ile, Arg, Asp, or Met;

Xaa at position 112 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;

10 Xaa at position 113 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp,
Lys, Leu, Ile, Val or Asn;

Xaa at position 114 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;

Xaa at position 115 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,
Trp, or Met;

15 Xaa at position 116 is Lys, Leu, Pro, Thr, Met, Asp, Val, Glu,
Arg, Trp, Ser, Asn, His, Ala, Tyr, Phe, Gln, or Ile;

Xaa at position 117 is Thr, Ser, Asn, Ile, Trp, Lys, or Pro;

Xaa at position 118 is Leu, Ser, Pro, Ala, Glu, Cys, Asp, or Tyr;

Xaa at position 119 is Glu, Ser, Lys, Pro, Leu, Thr, Tyr, or Arg;

20 Xaa at position 120 is Asn, Ala, Pro, Leu, His, Val, or Gln;

Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or
Gly;

Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,
Ile, Tyr, or Cys;

25 Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in
position 1; and wherein from 1 to 14 amino acids can be deleted
from the N-terminus and/or from 1 to 15 amino acids can be
30 deleted from the C-terminus; and wherein from 4 to 44 of the
amino acids designated by Xaa are different from the
corresponding amino acids of native (1-133) human interleukin-3.

Included in the present invention are human interleukin-3

35 mutant polypeptide of the Formula II:

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| | | | | | | | | | | | | | | | |
|----|---------|-----|----------|-----|-----|------|------|------|------|------|------|-----|------|---------|-----|
| | Ala | Pro | Met | Thr | Gln | Thr | Thr | Ser | Leu | Lys | Thr | Ser | Trp | Val | Asn |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 |
| | Cys | Xaa | Xaa | Xaa | Xaa | Xaa | Glu | Xaa | Xaa | Xaa | Xaa | Leu | Xaa | Xaa | Xaa |
| 5 | | | | | 20 | | | | | 25 | | | | | 30 |
| | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Asn | Leu | Xaa | Xaa | Glu | Xaa | Xaa |
| | | | | | 35 | | | | | 40 | | | | | 45 |
| 10 | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Asn | Leu | Xaa | Xaa |
| | | | | | 50 | | | | | 55 | | | | | 60 |
| | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa |
| 15 | | | | | 65 | | | | | 70 | | | | | 75 |
| | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Xaa | Xaa | Cys | Xaa | Pro | Xaa | Xaa | Xaa | Xaa |
| | | | | | 80 | | | | | 85 | | | | | 90 |
| 20 | Xaa | Xaa | Xaa | Arg | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Asp | Xaa | Xaa |
| | | | | | 95 | | | | | 100 | | | | | 105 |
| | Xaa | Phe | Xaa | Xaa | Lys | Leu | Xaa | Phe | Xaa | Xaa | Xaa | Xaa | Leu | Xaa | Xaa |
| | | | | | 110 | | | | | 115 | | | | | 120 |
| 25 | | | | | | | | | | | | | | | |
| | Xaa | Xaa | Xaa | Gln | Gln | Thr | Thr | Leu | Ser | Leu | Ala | Ile | Phe | [SEQ ID | |
| | | | | | | | | | | | | | | NO:16] | |
| | | | | | 125 | | | | | 130 | | | | | |
| 30 | wherein | | | | | | | | | | | | | | |
| | Xaa | at | position | 17 | is | Ser, | Gly, | Asp, | Met, | or | Gln; | | | | |
| | Xaa | at | position | 18 | is | Asn, | His, | Leu, | Ile, | Phe, | Arg, | or | Gln; | | |
| | Xaa | at | position | 19 | is | Met, | Phe, | Ile, | Arg, | or | Ala; | | | | |
| | Xaa | at | position | 20 | is | Ile | or | Pro; | | | | | | | |
| 35 | Xaa | at | position | 21 | is | Asp | or | Glu; | | | | | | | |
| | Xaa | at | position | 23 | is | Ile, | Val, | Ala, | Leu, | or | Gly; | | | | |

- Xaa at position 24 is Ile, Val, Phe, or Leu;
 Xaa at position 25 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
 Xaa at position 26 is His, Phe, Gly, Arg, or Ala;
 Xaa at position 28 is Lys, Leu, Gln, Gly, Pro, or Val;
 5 Xaa at position 29 is Gln, Asn, Leu, Arg, or Val;
 Xaa at position 30 is Pro, His, Thr, Gly, or Gln;
 Xaa at position 31 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
 Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
 Xaa at position 33 is Pro, Leu, Gln, Ala, or Glu;
 10 Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
 Glu,
 Ile, Phe, Thr or Met;
 Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
 Xaa at position 36 is Asp or Leu;
 15 Xaa at position 37 is Phe, Ser, Pro, Trp, or Ile;
 Xaa at position 38 is Asn or Ala;
 Xaa at position 41 is Asn, Cys, Arg, His, Met, or Pro;
 Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
 Met,
 20 Tyr, Val or Arg;
 Xaa at position 44 is Asp or Glu;
 Xaa at position 45 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn,
 Glu,
 Ser, or Trp;
 25 Xaa at position 46 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln,
 Glu,
 His, Ile, Lys, Tyr, Val or Gly;
 Xaa at position 47 is Ile, Val, or His;
 Xaa at position 49 is Met, Asn, or Asp;
 30 Xaa at position 50 is Glu, Thr, Ala, Asn, Ser or Asp;
 Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
 Xaa at position 52 is Asn or Gly;
 Xaa at position 53 is Leu, Met, or Phe;
 Xaa at position 54 is Arg, Ala, or Ser;
 35 Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
 Xaa at position 56 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn,

Glu, His,

Leu, Thr, Val or Lys;

Xaa at position 59 is Glu, Tyr, His, Leu, or Arg;

Xaa at position 60 is Ala, Ser, Asn, or Thr;

5 Xaa at position 61 is Phe or Ser;

Xaa at position 62 is Asn, Val, Pro, Thr, or Ile;

Xaa at position 63 is Arg, Tyr, Lys, Ser, His, or Val;

Xaa at position 64 is Ala or Asn;

Xaa at position 65 is Val, Thr, Leu, or Ser;

10 Xaa at position 66 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;

Xaa at position 67 is Ser, Phe, Val, Gly, Asn, Ile, or His;

Xaa at position 68 is Leu, Val, Ile, Phe, or His;

Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;

Xaa at position 70 is Asn or Pro;

15 Xaa at position 71 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;

Xaa at position 72 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;

Xaa at position 73 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or

Pro;

Xaa at position 74 is Ile or Met;

20 Xaa at position 75 is Glu, Gly, Asp, Ser, or Gln;

Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or

Asp;

Xaa at position 77 is Ile, Ser, or Leu;

Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or

25 Asp;

Xaa at position 80 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;

Xaa at position 81 is Leu, or Val;

Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,

His,

30 Met, Phe, Ser, Thr, Tyr or Val;

Xaa at position 83 is Pro, Ala, Thr, Trp, or Met;

Xaa at position 85 is Leu or Val;

Xaa at position 87 is Leu or Ser;

Xaa at position 88 is Ala, Arg, or Trp;

35 Xaa at position 89 is Thr, Asp, Glu, His, Asn, or Ser;

Xaa at position 90 is Ala, Asp, or Met;

- Xaa at position 91 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
Xaa at position 92 is Pro or Ser;
Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile,
5 Phe,
Ser or Thr;
Xaa at position 96 is Pro or Tyr;
Xaa at position 97 is Ile, Val, or Ala;
Xaa at position 98 is His, Ile, Asn, Leu, Asp, Ala, Thr, Leu,
10 Arg, Gln,
Glu, Lys, Met, Ser, Tyr, Val or Pro;
Xaa at position 99 is Ile, Leu, Val, or Phe;
Xaa at position 100 is Lys, Leu, His, Arg, Ile, Gln, Pro, or
Ser;
15 Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Val,
Asn, Ile, Leu or Tyr;
Xaa at position 102 is Gly, Glu, Lys, or Ser;
Xaa at position 104 is Trp, Val, Tyr, Met, or Leu;
Xaa at position 105 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
20 Leu, Lys, Ile, Asp, or His;
Xaa at position 106 is Glu, Ser, Ala, or Gly;
Xaa at position 108 is Arg, Ala, Gln, Ser or Lys;
Xaa at position 109 is Arg, Thr, Glu, Leu, Ser, or Gly;
Xaa at position 112 is Thr, Val, Gln, Glu, His, or Ser;
25 Xaa at position 114 is Tyr or Trp;
Xaa at position 115 is Leu or Ala;
Xaa at position 116 is Lys, Thr, Met, Val, Trp, Ser, Leu, Ala,
Asn,
Gln, His, Met, Phe, Tyr or Ile;
30 Xaa at position 117 is Thr, Ser, or Asn;
Xaa at position 119 is Glu, Ser, Pro, Leu, Thr, or Tyr;
Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or
Gly;
35 Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,
Ile, Tyr, or Cys;

20

Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from 4 to 44 of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3.

10 Included in the present invention are human interleukin-3 mutant polypeptide of the Formula III:

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Ala | Pro | Met | Thr | Gln | Thr | Thr | Ser | Leu | Lys | Thr | Ser | Trp | Val | Asn |
| | 1 | | | | 5 | | | | | 10 | | | | | 15 |
| 15 | Cys | Xaa | Xaa | Xaa | Ile | Xaa | Glu | Xaa | Xaa | Xaa | Xaa | Leu | Lys | Xaa | Xaa |
| | | | | | 20 | | | | | 25 | | | | | 30 |
| | Xaa | Xaa | Xaa | Xaa | Xaa | Asp | Xaa | Xaa | Asn | Leu | Asn | Xaa | Glu | Xaa | Xaa |
| 20 | | | | | 35 | | | | | 40 | | | | | 45 |
| | Xaa | Ile | Leu | Met | Xaa | Xaa | Asn | Leu | Xaa | Xaa | Xaa | Asn | Leu | Glu | Xaa |
| | | | | | 50 | | | | | 55 | | | | | 60 |
| 25 | Phe | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Asn | Xaa | Xaa | Xaa | Ile | Glu |
| | | | | | 65 | | | | | 70 | | | | | 75 |
| | Xaa | Xaa | Leu | Xaa | Xaa | Leu | Xaa | Xaa | Cys | Xaa | Pro | Xaa | Xaa | Thr | Ala |
| | | | | | 80 | | | | | 85 | | | | | 90 |
| 30 | Xaa | Pro | Xaa | Arg | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Gly | Asp | Xaa | Xaa |
| | | | | | 95 | | | | | 100 | | | | | 105 |
| | Xaa | Phe | Xaa | Xaa | Lys | Leu | Xaa | Phe | Xaa | Xaa | Xaa | Xaa | Leu | Glu | Xaa |
| 35 | | | | | 110 | | | | | 115 | | | | | 120 |

21

Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID
NO:17]

125

130

- 5 wherein
- Xaa at position 17 is Ser, Gly, Asp, Met, or Gln;
- Xaa at position 18 is Asn, His, or Ile;
- Xaa at position 19 is Met or Ile;
- Xaa at position 21 is Asp or Glu;
- 10 Xaa at position 23 is Ile, Ala, Leu, or Gly;
- Xaa at position 24 is Ile, Val, or Leu;
- Xaa at position 25 is Thr, His, Gln, or Ala;
- Xaa at position 26 is His or Ala;
- Xaa at position 29 is Gln, Asn, or Val;
- 15 Xaa at position 30 is Pro, Gly, or Gln;
- Xaa at position 31 is Pro, Asp, Gly, or Gln;
- Xaa at position 32 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
- Xaa at position 33 is Pro or Glu;
- Xaa at position 34 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
- 20 Glu, Ile, Phe, Thr or Met;
- Xaa at position 35 is Leu, Ala, Asn, Pro, Gln, or Val;
- Xaa at position 37 is Phe, Ser, Pro, or Trp;
- Xaa at position 38 is Asn or Ala;
- Xaa at position 42 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
- 25 Met, Tyr or Arg;
- Xaa at position 44 is Asp or Glu;
- Xaa at position 45 is Gln, Val, Met, Leu, Thr, Ala, Asn, Glu,
Ser or Lys;
- Xaa at position 46 is Asp, Phe, Ser, Thr, Ala, Asn Gln, Glu, His,
- 30 Ile, Lys, Tyr, Val or Cys;
- Xaa at position 50 is Glu, Ala, Asn, Ser or Asp;
- Xaa at position 51 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- Xaa at position 54 is Arg or Ala;
- Xaa at position 54 is Arg or Ala;
- 35 Xaa at position 55 is Arg, Thr, Val, Leu, or Gly;
- Xaa at position 56 is Pro, Gly, Ser, Gln, Ala, Arg, Asn, Glu,

- Leu, Thr, Val or Lys;
Xaa at position 60 is Ala or Ser;
Xaa at position 62 is Asn, Pro, Thr, or Ile;
Xaa at position 63 is Arg or Lys;
5 Xaa at position 64 is Ala or Asn;
Xaa at position 65 is Val or Thr;
Xaa at position 66 is Lys or Arg;
Xaa at position 67 is Ser, Phe, or His;
Xaa at position 68 is Leu, Ile, Phe, or His;
10 Xaa at position 69 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 71 is Ala, Pro, or Arg;
Xaa at position 72 is Ser, Glu, Arg, or Asp;
Xaa at position 73 is Ala or Leu;
Xaa at position 76 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;
15 Xaa at position 77 is Ile or Leu;
Xaa at position 79 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly, or
Asp;
Xaa at position 80 is Asn, Gly, Glu, or Arg;
Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,
20 His,
Ile, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 83 is Pro or Thr;
Xaa at position 85 is Leu or Val;
Xaa at position 87 is Leu or Ser;
25 Xaa at position 88 is Ala or Trp;
Xaa at position 91 is Ala or Pro;
Xaa at position 93 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 95 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe, Ser
or Thr;
30 Xaa at position 96 is Pro or Tyr;
Xaa at position 97 is Ile or Val;
Xaa at position 98 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg,
Gln,
Leu, Lys, Met, Ser, Tyr, Val or Pro;
35 Xaa at position 99 is Ile, Leu, or Val;
Xaa at position 100 is Lys, Arg, Ile, Gln, Pro, or Ser;

- Xaa at position 101 is Asp, Pro, Met, Lys, His, Thr, Pro, Asn, Ile, Leu or Tyr;
 Xaa at position 104 is Trp or Leu;
 Xaa at position 105 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,
 5 Lys, Ile, Asp, or His;
 Xaa at position 106 is Glu or Gly;
 Xaa at position 108 is Arg, Ala, or Ser;
 Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
 Xaa at position 112 is Thr, Val, or Gln;
 10 Xaa at position 114 is Tyr or Trp;
 Xaa at position 115 is Leu or Ala;
 Xaa at position 116 is Lys, Thr, Val, Trp, Ser, Ala, His, Met, Phe, Tyr or Ile;
 Xaa at position 117 is Thr or Ser;
 15 Xaa at position 120 is Asn, Pro, Leu, His, Val, or Gln;
 Xaa at position 121 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
 Xaa at position 122 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His, Ile, Tyr, or Cys;
 Xaa at position 123 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;
 20
 and which can additionally have Met- preceding the amino acid in position 1; and wherein from 1 to 14 amino acids can be deleted from the N-terminus and/or from 1 to 15 amino acids can be deleted from the C-terminus; and wherein from 4 to 35 of the
 25 amino acids designated by Xaa are different from the corresponding amino acids of native (1-133)human interleukin-3.

Included in the present invention are human interleukin-3 mutant polypeptide of the Formula IV:

30

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Met | Thr | Gln | Thr | Thr | Ser | Leu | Lys | Thr | Ser | Trp | Val | Asn |
| 1 | | | | 5 | | | | | 10 | | | | 15 | |

| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Xaa | Xaa | Met | Ile | Asp | Glu | Xaa | Ile | Xaa | Xaa | Leu | Lys | Xaa | Xaa |
| 35 | | | | 20 | | | | 25 | | | | 30 | | |

24

Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp Xaa
 35 40 45
 Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu Ala
 5 50 55 60
 Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile Glu
 65 70 75
 10 Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr Ala
 80 85 90
 Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp Xaa
 95 100 105
 15 Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu Xaa
 110 115 120
 Xaa Xaa Xaa Gln Gln Thr Thr Leu Ser Leu Ala Ile Phe [SEQ ID
 20 NO:18]
 125 130
 wherein
 Xaa at position 17 is Ser, Gly, Asp, or Gln;
 Xaa at position 18 is Asn, His, or Ile;
 25 Xaa at position 23 is Ile, Ala, Leu, or Gly;
 Xaa at position 25 is Thr, His, or Gln;
 Xaa at position 26 is His or Ala;
 Xaa at position 29 is Gln or Asn;
 Xaa at position 30 is Pro or Gly;
 30 Xaa at position 32 is Leu, Arg, Asn, or Ala;
 Xaa at position 34 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,
 Phe, Thr, or Met;
 Xaa at position 35 is Leu, Ala, Asn, or Pro;
 Xaa at position 38 is Asn or Ala;
 35 Xaa at position 42 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met,
 Tyr or Arg;

25

- Xaa at position 45 is Gln, Val, Met, Leu, Ala, Asn, Glu, or Lys;
 Xaa at position 46 is Asp, Phe, Ser, Gln, Glu, His, Val
 or Thr;
 Xaa at position 50 is Glu Asn, Ser or Asp;
- 5 Xaa at position 51 is Asn, Arg, Pro, Thr, or His;
 Xaa at position 55 is Arg, Leu, or Gly;
 Xaa at position 56 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;
 Xaa at position 62 is Asn, Pro, or Thr;
 Xaa at position 64 is Ala or Asn;
- 10 Xaa at position 65 is Val or Thr;
 Xaa at position 67 is Ser or Phe;
 Xaa at position 68 is Leu or Phe;
 Xaa at position 69 is Gln, Ala, Glu, or Arg;
 Xaa at position 76 is Ser, Val, Asn, Pro, or Gly;
- 15 Xaa at position 77 is Ile or Leu;
 Xaa at position 79 is Lys, Gly, Asn, Met, Arg, Ile, or Gly;
 Xaa at position 80 is Asn, Gly, Glu, or Arg;
 Xaa at position 82 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His,
 Met,
- 20 Phe, Ser, Thr, Tyr or Val;
 Xaa at position 87 is Leu or Ser;
 Xaa at position 88 is Ala or Trp;
 Xaa at position 91 is Ala or Pro;
 Xaa at position 93 is Thr, Asp, or Ala;
- 25 Xaa at position 95 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
 Xaa at position 98 is His, Ile, Asn, Ala, Thr, Gln, Glu,
 Lys, Met, Ser, Tyr, Val or Leu;
 Xaa at position 99 is Ile or Leu;
 Xaa at position 100 is Lys or Arg;
- 30 Xaa at position 101 is Asp, Pro, Met, Lys, Thr, His, Pro, Asn,
 Ile,
 Leu or Tyr;
 Xaa at position 105 is Asn, Pro, Ser, Ile or Asp;
 Xaa at position 108 is Arg, Ala, or Ser;
- 35 Xaa at position 109 is Arg, Thr, Glu, Leu, or Ser;
 Xaa at position 112 is Thr or Gln;

Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:19]

110

5

wherein

Xaa at position 3 is Ser, Lys, Gly, Asp, Met, Gln, or Arg;

Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;

Xaa at position 5 is Met, Phe, Ile, Arg, Gly, Ala, or Cys;

10 Xaa at position 6 is Ile, Cys, Gln, Glu, Arg, Pro, or Ala;

Xaa at position 7 is Asp, Phe, Lys, Arg, Ala, Gly, Glu, Gln, Asn,
Thr, Ser or Val;

Xaa at position 8 is Glu, Trp, Pro, Ser, Ala, His, Asp, Asn, Gln,
Leu, Val, or Gly;

15 Xaa at position 9 is Ile, Val, Ala, Leu, Gly, Trp, Lys, Phe,
Leu, Ser, or Arg;

Xaa at position 10 is Ile, Gly, Val, Arg, Ser, Phe, or Leu;

Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;

Xaa at position 12 is His, Thr, Phe, Gly, Arg, Ala, or Trp;

20 Xaa at position 13 is Leu, Gly, Arg, Thr, Ser, or Ala;

Xaa at position 14 is Lys, Arg, Leu, Gln, Gly, Pro, Val or Trp;

Xaa at position 15 is Gln, Asn, Leu, Pro, Arg, or Val;

Xaa at position 16 is Pro, His, Thr, Gly, Asp, Gln, Ser, Leu, or
Lys;

25 Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;

Xaa at position 18 is Leu, Val, Arg, Gln, Asn, Gly, Ala, or Glu;

Xaa at position 19 is Pro, Leu, Gln, Ala, Thr, or Glu;

Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Glu, Gln, Thr,
Arg, Ala, Phe, Ile or Met;

30 Xaa at position 21 is Leu, Ala, Gly, Asn, Pro, Gln, or Val;

Xaa at position 22 is Asp, Leu, or Val;

Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;

Xaa at position 24 is Asn, or Ala;

Xaa at position 26 is Leu, Trp, or Arg;

35 Xaa at position 27 is Asn, Cys, Arg, Leu, His, Met, Pro;

Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Lys, Asn, Thr,

- Leu,
 Val, Glu, Phe, Tyr, Ile or Met;
 Xaa at position 29 is Glu, Asn, Tyr, Leu, Phe, Asp, Ala, Cys,
 Gln,
- 5 Arg, Thr, Gly or Ser;
 Xaa at position 30 is Asp, Ser, Leu, Arg, Lys, Thr, Met, Trp, Glu,
 Asn, Gln, Ala or Pro;
 Xaa at position 31 is Gln, Pro, Phe, Val, Met, Leu, Thr, Lys,
 Asp,
- 10 Asn, Arg, Ser, Ala, Ile, Glu, His or Trp;
 Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Glu, Asn, Gln,
 Lys, His, Ala, Tyr, Ile, Val or Gly;
 Xaa at position 33 is Ile, Gly, Val, Ser, Arg, Pro, or His;
 Xaa at position 34 is Leu, Ser, Cys, Arg, Ile, His, Phe, Glu,
- 15 Lys, Thr, Ala, Met, Val or Asn;
 Xaa at position 35 is Met, Arg, Ala, Gly, Pro, Asn, His, or Asp;
 Xaa at position 36 is Glu, Leu, Thr, Asp, Tyr, Lys, Asn, Ser,
 Ala,
 Ile, Val, His, Phe, Met or Gln;
- 20 Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
 Xaa at position 38 is Asn, His, Arg, Leu, Gly, Ser, or Thr;
 Xaa at position 39 is Leu, Thr, Ala, Gly, Glu, Pro, Lys, Ser,
 Met, or;
 Xaa at position 40 is Arg, Asp, Ile, Ser, Val, Thr, Gln, Asn,
- 25 Lys, His, Ala or Leu;
 Xaa at position 41 is Arg, Thr, Val, Ser, Leu, or Gly;
 Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Glu, Arg, His,
 Thr, Ala, Tyr, Phe, Leu, Val or Lys;
 Xaa at position 43 is Asn or Gly;
- 30 Xaa at position 44 is Leu, Ser, Asp, Arg, Gln, Val, or Cys;
 Xaa at position 45 is Glu Tyr, His, Leu, Pro, or Arg;
 Xaa at position 46 is Ala, Ser, Pro, Tyr, Asn, or Thr;
 Xaa at position 47 is Phe, Asn, Glu, Pro, Lys, Arg, or Ser;
 Xaa at position 48 is Asn, His, Val, Arg, Pro, Thr, Asp, or Ile;
- 35 Xaa at position 49 is Arg, Tyr, Trp, Lys, Ser, His, Pro, or Val;
 Xaa at position 50 is Ala, Asn, Pro, Ser, or Lys;

- Xaa at position 51 is Val, Thr, Pro, His, Leu, Phe, or Ser;
Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
Xaa at position 53 is Ser, Ala, Phe, Val, Gly, Asn, Ile, Pro, or His;
- 5 Xaa at position 54 is Leu, Val, Trp, Ser, Ile, Phe, Thr, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, Trp, Gly, or Leu;
- Xaa at position 56 is Asn, Leu, Val, Trp, Pro, or Ala;
Xaa at position 57 is Ala, Met, Leu, Pro, Arg, Glu, Thr, Gln,
- 10 Trp, or Asn;
- Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, or Arg;
Xaa at position 60 is Ile, Met, Thr, Pro, Arg, Gly, Ala;
Xaa at position 61 is Glu, Lys, Gly, Asp, Pro, Trp, Arg, Ser,
- 15 Gln, or Leu;
- Xaa at position 62 is Ser, Val, Ala, Asn, Trp, Glu, Pro, Gly, or Asp;
- Xaa at position 63 is Ile, Ser, Arg, Thr, or Leu;
Xaa at position 64 is Leu, Ala, Ser, Glu, Phe, Gly, or Arg;
- 20 Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or Asp;
- Xaa at position 66 is Asn, Trp, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, Gln, Gly, Ala, Trp, Arg, Val, or Lys;
Xaa at position 68 is Leu, Gln, Lys, Trp, Arg, Asp, Glu, Asn,
- 25 His, Thr, Ser, Ala, Tyr, Phe, Ile, Met or Val;
- Xaa at position 69 is Pro, Ala, Thr, Trp, Arg, or Met;
Xaa at position 70 is Cys, Glu, Gly, Arg, Met, or Val;
Xaa at position 71 is Leu, Asn, Val, or Gln;
Xaa at position 72 is Pro, Cys, Arg, Ala, or Lys;
- 30 Xaa at position 73 is Leu, Ser, Trp, or Gly;
Xaa at position 74 is Ala, Lys, Arg, Val, or Trp;
Xaa at position 75 is Thr, Asp, Cys, Leu, Val, Glu, His, Asn, or Ser;
- Xaa at position 76 is Ala, Pro, Ser, Thr, Gly, Asp, Ile, or Met;
- 35 Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, Asp, or His;
Xaa at position 78 is Pro, Phe, Arg, Ser, Lys, His, Ala, Gly, Ile

or Leu;

Xaa at position 79 is Thr, Asp, Ser, Asn, Pro, Ala, Leu, or Arg;

Xaa at position 80 is Arg, Ile, Ser, Glu, Leu, Val, Gln, Lys,
His,

5 Ala or Pro;

Xaa at position 81 is His, Gln, Pro, Arg, Val, Leu, Gly, Thr,
Asn,

Lys, Ser, Ala, Trp, Phe, Ile or Tyr;

Xaa at position 82 is Pro, Lys, Tyr, Gly, Ile, or Thr;

10 Xaa at position 83 is Ile, Val, Lys, Ala, or Asn;

Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr, Glu,
Gln, Ser, Phe, Met, Val, Lys, Arg, Tyr or Pro;

Xaa at position 85 is Ile, Leu, Arg, Asp, Val, Pro, Gln,
Gly, Ser, Phe, or His;

15 Xaa at position 86 is Lys, Tyr, Leu, His, Arg, Ile, Ser, Gln,
Pro;

Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val,

Tyr, Glu, Asn, Ser, Ala, Gly, Ile, Leu or Gln;

Xaa at position 88 is Gly, Leu, Glu, Lys, Ser, Tyr, or Pro;

20 Xaa at position 89 is Asp, or Ser;

Xaa at position 90 is Trp, Val, Cys, Tyr, Thr, Met, Pro, Leu,
Gln, Lys, Ala, Phe, or Gly;

Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
Leu, Lys, Ile, Asp, or His;

25 Xaa at position 92 is Glu, Ser, Ala, Lys, Thr, Ile, Gly, or Pro;

Xaa at position 94 is Arg, Lys, Asp, Leu, Thr, Ile, Gln,
His, Ser, Ala, or Pro;

Xaa at position 95 is Arg, Thr, Pro, Glu, Tyr, Leu, Ser, or Gly;

Xaa at position 96 is Lys, Asn, Thr, Leu, Gln, Arg,

30 His, Glu, Ser, Ala or Trp;

Xaa at position 97 is Leu, Ile, Arg, Asp, or Met;

Xaa at position 98 is Thr, Val, Gln, Tyr, Glu, His, Ser, or Phe;

Xaa at position 99 is Phe, Ser, Cys, His, Gly, Trp, Tyr, Asp,
Lys, Leu, Ile, Val or Asn;

35 Xaa at position 100 is Tyr, Cys, His, Ser, Trp, Arg, or Leu;

Xaa at position 101 is Leu, Asn, Val, Pro, Arg, Ala, His, Thr,

32

Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa
 65 70 75
 Xaa Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa
 5 80 85 90
 Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Xaa
 95 100 105
 10 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:20]
 110

wherein

- 15 Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
 Xaa at position 4 is Asn, His, Leu, Ile, Phe, Arg, or Gln;
 Xaa at position 5 is Met, Phe, Ile, Arg, or Ala;
 Xaa at position 6 is Ile or Pro;
 Xaa at position 7 is Asp, or Glu;
 20 Xaa at position 9 is Ile, Val, Ala, Leu, or Gly;
 Xaa at position 10 is Ile, Val, Phe, or Leu;
 Xaa at position 11 is Thr, His, Gly, Gln, Arg, Pro, or Ala;
 Xaa at position 12 is His, Phe, Gly, Arg, or Ala;
 Xaa at position 14 is Lys, Leu, Gln, Gly, Pro, or Val;
 25 Xaa at position 15 is Gln, Asn, Leu, Arg, or Val;
 Xaa at position 16 is Pro, His, Thr, Gly, or Gln;
 Xaa at position 17 is Pro, Asp, Gly, Ala, Arg, Leu, or Gln;
 Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
 Xaa at position 19 is Pro, Leu, Gln, Ala, or Glu;
 30 Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg, Gln,
 Glu, Ile, Phe, Thr or Met;
 Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
 Xaa at position 22 is Asp or Leu;
 Xaa at position 23 is Phe, Ser, Pro, Trp, or Ile;
 35 Xaa at position 24 is Asn or Ala;
 Xaa at position 27 is Asn, Cys, Arg, His, Met, or Pro;

- Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile, Leu,
Met, Tyr, or Arg;
- Xaa at position 30 is Asp, or Glu;
- Xaa at position 31 is Gln, Val, Met, Leu, Thr, Lys, Ala, Asn Glu,
5 Ser or Trp;
- Xaa at position 32 is Asp, Phe, Ser, Thr, Cys, Ala, Asn, Gln,
Glu, His, Ile, Lys, Tyr, Val or Gly;
- Xaa at position 33 is Ile, Val, or His;
- Xaa at position 35 is Met, Asn, or Asp;
- 10 Xaa at position 36 is Glu, Thr, Ala, Asn, Ser or Asp;
- Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
- Xaa at position 38 is Asn or Gly;
- Xaa at position 39 is Leu, Met, or Phe;
- Xaa at position 40 is Arg, Ala or Ser;
- 15 Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
- Xaa at position 42 is Pro, Gly, Cys, Ser, Gln, Ala, Arg, Asn,
Glu, His, Leu, Thr, Val or Lys;
- Xaa at position 45 is Glu, Tyr, His, Leu, or Arg;
- Xaa at position 46 is Ala, Ser, Asn, or Thr;
- 20 Xaa at position 47 is Phe or Ser;
- Xaa at position 48 is Asn, Val, Pro, Thr, or Ile;
- Xaa at position 49 is Arg, Tyr, Lys, Ser, His, or Val;
- Xaa at position 50 is Ala or Asn;
- Xaa at position 51 is Val, Thr, Leu, or Ser;
- 25 Xaa at position 52 is Lys, Ile, Arg, Val, Asn, Glu, or Ser;
- Xaa at position 53 is Ser, Phe, Val, Gly, Asn, Ile, or His;
- Xaa at position 54 is Leu, Val, Ile, Phe, or His;
- Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
- Xaa at position 56 is Asn or Pro;
- 30 Xaa at position 57 is Ala, Met, Pro, Arg, Glu, Thr, or Gln;
- Xaa at position 58 is Ser, Glu, Met, Ala, His, Asn, Arg, or Asp;
- Xaa at position 59 is Ala, Glu, Asp, Leu, Ser, Gly, Thr, Arg, or
Pro;
- Xaa at position 60 is Ile or Met;
- 35 Xaa at position 61 is Glu, Gly, Asp, Ser, or Gln;
- Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, Gly, or

- Asp;
Xaa at position 63 is Ile, Ser, or Leu;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, or
Asp;
5 Xaa at position 66 is Asn, Val, Gly, Thr, Leu, Glu, or Arg;
Xaa at position 67 is Leu, or Val;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,
His, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 69 is Pro, Ala, Thr, Trp, or Met;
10 Xaa at position 71 is Leu or Val;
Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala, Arg, or Trp;
Xaa at position 75 is Thr, Asp, Glu, His, Asn, or Ser;
Xaa at position 76 is Ala, Asp, or Met;
15 Xaa at position 77 is Ala, Pro, Ser, Thr, Phe, Leu, or Asp;
Xaa at position 78 is Pro or Ser;
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Ile,
Phe,
20 Ser or Thr;
Xaa at position 82 is Pro or Tyr;
Xaa at position 83 is Ile, Val, or Ala;
Xaa at position 84 is His, Ile, Asn, Leu, Asp, Ala, Thr,
Arg, Gln, Glu, Lys, Met, Ser, Tyr, Val or Pro;
25 Xaa at position 85 is Ile, Leu, Val, or Phe;
Xaa at position 86 is Lys, Leu, His, Arg, Ile, Gln, Pro or
Ser;
Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Val,
Asn, Ile, Leu or Tyr;
30 Xaa at position 88 is Gly, Glu, Lys, or Ser;
Xaa at position 90 is Trp, Val, Tyr, Met, or Leu;
Xaa at position 91 is Asn, Pro, Ala, Phe, Ser, Trp, Gln, Tyr,
Leu, Lys, Ile, Asp, or His;
Xaa at position 92 is Glu, Ser, Ala, or Gly;
35 Xaa at position 94 is Arg, Ala, Gln, Ser or Lys;
Xaa at position 95 is Arg, Thr, Glu, Leu, Ser, or Gly;

Xaa at position 98 is Thr, Val, Gln, Glu, His, or Ser;

Xaa at position 100 is Tyr or Trp;

Xaa at position 101 is Leu or Ala;

Xaa at position 102 is Lys, Thr, Met, Val, Trp, Ser, Leu,

5 Ala, Asn, Gln, His, Met, Phe, Tyr or Ile;

Xaa at position 103 is Thr, Ser, or Asn;

Xaa at position 105 is Glu, Ser, Pro, Leu, Thr, or Tyr;

Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Lys, Asp, or

10 Gly;

Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,

Ile, Tyr, or Cys;

Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;

15 and which can additionally have Met- or Met-Ala- preceding the amino acid in position 1; and wherein from 4 to 44 of the amino acids designated by Xaa are different from the corresponding amino acids of native (1-133) human interleukin-3; or a polypeptide having substantially the same structure and
20 substantially the same biological activity.

Included in the present invention are (15-125)human interleukin-3 mutant polypeptides of the Formula VII:

```

25  Asn Cys Xaa Xaa Xaa Ile Xaa Glu Xaa Xaa Xaa Xaa Leu Lys Xaa
      1             5             10             15

```

Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa Xaa Asn Leu Asn Xaa Glu Xaa
20 25 30

30 Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Xaa Xaa Xaa Asn Leu Glu
35 40 45

35 Xaa Phe Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn Xaa Xaa Xaa Ile
50 55 60

36

Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Xaa Cys Xaa Pro Xaa Xaa Thr
 65 70 75

 Ala Xaa Pro Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Asp Xaa
 5 80 85 90

 Xaa Xaa Phe Xaa Xaa Lys Leu Xaa Phe Xaa Xaa Xaa Xaa Leu Glu
 95 100 105

 10 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:21]
 110

wherein

- Xaa at position 3 is Ser, Gly, Asp, Met, or Gln;
 15 Xaa at position 4 is Asn, His, or Ile;
 Xaa at position 5 is Met or Ile;
 Xaa at position 7 is Asp or Glu;
 Xaa at position 9 is Ile, Ala, Leu, or Gly;
 Xaa at position 10 is Ile, Val, or Leu;
 20 Xaa at position 11 is Thr, His, Gln, or Ala;
 Xaa at position 12 is His or Ala;
 Xaa at position 15 is Gln, Asn, or Val;
 Xaa at position 16 is Pro, Gly, or Gln;
 Xaa at position 17 is Pro, Asp, Gly, or Gln;
 25 Xaa at position 18 is Leu, Arg, Gln, Asn, Gly, Ala, or Glu;
 Xaa at position 19 is Pro or Glu;
 Xaa at position 20 is Leu, Val, Gly, Ser, Lys, Ala, Arg,
 Gln, Glu, Ile, Phe, Thr or Met;
 Xaa at position 21 is Leu, Ala, Asn, Pro, Gln, or Val;
 30 Xaa at position 23 is Phe, Ser, Pro, or Trp;
 Xaa at position 24 is Asn or Ala;
 Xaa at position 28 is Gly, Asp, Ser, Cys, Ala, Asn, Ile,
 Leu, Met Tyr or Arg;
 Xaa at position 30 is Asp or Glu;
 35 Xaa at position 31 is Gln, Val, Met, Leu, Thr, Ala, Asn,
 Glu, Ser or Lys;

- Xaa at position 32 is Asp, Phe, Ser, Thr, Ala, Asn, Gln, Glu,
His, Ile, Lys, Tyr, Val or Cys;
Xaa at position 36 is Glu, Ala, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Met, Pro, Ser, Thr, or His;
5 Xaa at position 40 is Arg or Ala;
Xaa at position 41 is Arg, Thr, Val, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Ser, Gln, Ala, Arg, Asn,
Glu, Leu, Thr, Val or Lys;
Xaa at position 46 is Ala or Ser;
10 Xaa at position 48 is Asn, Pro, Thr, or Ile;
Xaa at position 49 is Arg or Lys;
Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val or Thr;
Xaa at position 52 is Lys or Arg;
15 Xaa at position 53 is Ser, Phe, or His;
Xaa at position 54 is Leu, Ile, Phe, or His;
Xaa at position 55 is Gln, Ala, Pro, Thr, Glu, Arg, or Gly;
Xaa at position 57 is Ala, Pro, or Arg;
Xaa at position 58 is Ser, Glu, Arg, or Asp;
20 Xaa at position 59 is Ala or Leu;
Xaa at position 62 is Ser, Val, Ala, Asn, Glu, Pro, or Gly;
Xaa at position 63 is Ile or Leu;
Xaa at position 65 is Lys, Thr, Gly, Asn, Met, Arg, Ile, Gly,
or Asp;
25 Xaa at position 66 is Asn, Gly, Glu, or Arg;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Ala, Asn, Glu,
His, Ile, Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 69 is Pro or Thr;
Xaa at position 71 is Leu or Val;
30 Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala or Trp;
Xaa at position 77 is Ala or Pro;
Xaa at position 79 is Thr, Asp, Ser, Pro, Ala, Leu, or Arg;
Xaa at position 81 is His, Pro, Arg, Val, Leu, Gly, Asn, Phe,
35 Ser or Thr;
Xaa at position 82 is Pro or Tyr;

- Xaa at position 83 is Ile or Val;
 Xaa at position 84 is His, Ile, Asn, Leu, Ala, Thr, Leu, Arg,
 Gln, Leu, Lys, Met, Ser, Tyr, Val or Pro;
 Xaa at position 85 is Ile, Leu, or Val;
 5 Xaa at position 86 is Lys, Arg, Ile, Gln, Pro, or Ser;
 Xaa at position 87 is Asp, Pro, Met, Lys, His, Thr, Asn, Ile,
 Leu or Tyr;
 Xaa at position 90 is Trp or Leu;
 Xaa at position 91 is Asn, Pro, Ala, Ser, Trp, Gln, Tyr, Leu,
 10 Lys, Ile, Asp, or His;
 Xaa at position 92 is Glu, or Gly;
 Xaa at position 94 is Arg, Ala, or Ser;
 Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;
 Xaa at position 98 is Thr, Val, or Gln;
 15 Xaa at position 100 is Tyr or Trp;
 Xaa at position 101 is Leu or Ala;
 Xaa at position 102 is Lys, Thr, Val, Trp, Ser, Ala, His,
 Met, Phe, Tyr or Ile;
 Xaa at position 103 is Thr or Ser;
 20 Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;
 Xaa at position 107 is Ala, Ser, Ile, Asn, Pro, Asp, or Gly;
 Xaa at position 108 is Gln, Ser, Met, Trp, Arg, Phe, Pro, His,
 Ile, Tyr, or Cys;
 Xaa at position 109 is Ala, Met, Glu, His, Ser, Pro, Tyr, or Leu;
 25
- which can additionally have Met- or Met-Ala- preceding the amino
 acid in position 1; and wherein from 4 to 35 of the amino acids
 designated by Xaa are different from the corresponding amino
 acids of native human interleukin-3.

30

Included in the present invention are (15-125)human
 interleukin-3 mutant polypeptides of the Formula VIII:

35 Asn Cys Xaa Xaa Met Ile Asp Glu Xaa Ile Xaa Xaa Leu Lys Xaa
 1 5 10 15

Xaa Pro Xaa Pro Xaa Xaa Asp Phe Xaa Asn Leu Asn Xaa Glu Asp
 20 25 30

5 Xaa Xaa Ile Leu Met Xaa Xaa Asn Leu Arg Xaa Xaa Asn Leu Glu
 35 40 45

Ala Phe Xaa Arg Xaa Xaa Lys Xaa Xaa Xaa Asn Ala Ser Ala Ile
 50 55 60

10 Glu Xaa Xaa Leu Xaa Xaa Leu Xaa Pro Cys Leu Pro Xaa Xaa Thr
 65 70 75

Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Xaa Xaa Xaa Gly Asp Trp
 15 80 85 90

Xaa Glu Phe Xaa Xaa Lys Leu Xaa Phe Tyr Leu Xaa Xaa Leu Glu
 95 100 105

20 Xaa Xaa Xaa Xaa Gln Gln [SEQ ID NO:22]
 110

wherein

Xaa at position 3 is Ser, Gly, Asp, or Gln;

25 Xaa at position 4 is Asn, His, or Ile;

Xaa at position 9 is Ile, Ala, Leu, or Gly;

Xaa at position 11 is Thr, His, or Gln;

Xaa at position 12 is His or Ala;

Xaa at position 15 is Gln or Asn;

30 Xaa at position 16 is Pro or Gly;

Xaa at position 18 is Leu, Arg, Asn, or Ala;

Xaa at position 20 is Leu, Val, Ser, Ala, Arg, Gln, Glu, Ile,
 Phe, Thr or Met;

Xaa at position 21 is Leu, Ala, Asn, or Pro;

35 Xaa at position 24 is Asn or Ala;

Xaa at position 28 is Gly, Asp, Ser, Ala, Asn, Ile, Leu, Met,

Tyr or Arg;

Xaa at position 31 is Gln, Val, Met, Leu, Ala, Asn, Glu or Lys;

Xaa at position 32 is Asp, Phe, Ser, Ala, Gln, Glu, His, Val

or Thr;

- 5 Xaa at position 36 is Glu, Asn, Ser or Asp;
Xaa at position 37 is Asn, Arg, Pro, Thr, or His;
Xaa at position 41 is Arg, Leu, or Gly;
Xaa at position 42 is Pro, Gly, Ser, Ala, Asn, Val, Leu or Gln;
Xaa at position 48 is Asn, Pro, or Thr;
- 10 Xaa at position 50 is Ala or Asn;
Xaa at position 51 is Val or Thr;
Xaa at position 53 is Ser or Phe;
Xaa at position 54 is Leu or Phe;
Xaa at position 55 is Gln, Ala, Glu, or Arg;
- 15 Xaa at position 62 is Ser, Val, Asn, Pro, or Gly;
Xaa at position 63 is Ile or Leu;
Xaa at position 65 is Lys, Asn, Met, Arg, Ile, or Gly;
Xaa at position 66 is Asn, Gly, Glu, or Arg;
Xaa at position 68 is Leu, Gln, Trp, Arg, Asp, Asn, Glu, His,
- 20 Met, Phe, Ser, Thr, Tyr or Val;
Xaa at position 73 is Leu or Ser;
Xaa at position 74 is Ala or Trp;
Xaa at position 77 is Ala or Pro;
Xaa at position 79 is Thr, Asp, or Ala;
- 25 Xaa at position 81 is His, Pro, Arg, Val, Gly, Asn, Ser or Thr;
Xaa at position 84 is His, Ile, Asn, Ala, Thr, Arg, Gln, Glu,
Lys, Met, Ser, Tyr, Val or Leu;
Xaa at position 85 is Ile or Leu;
Xaa at position 86 is Lys or Arg;
- 30 Xaa at position 87 is Asp, Pro, Met, Lys, His, Pro, Asn, Ile,
Leu or Tyr;
Xaa at position 91 is Asn, Pro, Ser, Ile or Asp;
Xaa at position 94 is Arg, Ala, or Ser;
Xaa at position 95 is Arg, Thr, Glu, Leu, or Ser;
- 35 Xaa at position 98 is Thr or Gln;
Xaa at position 102 is Lys, Val, Trp, or Ile;

41

Xaa at position 103 is Thr, Ala, His, Phe, Tyr or Ser;

Xaa at position 106 is Asn, Pro, Leu, His, Val, or Gln;

Xaa at position 107 is Ala, Ser, Ile, Pro, or Asp;

Xaa at position 108 is Gln, Met, Trp, Phe, Pro, His, Ile, or

5 Tyr;

Xaa at position 109 is Ala, Met, Glu, Ser, or Leu;

and which can additionally have Met- or Met-Ala- preceding the
amino acid in position 1; and wherein from 4 to 26 of the amino
10 acids designated by Xaa are different from the corresponding
amino acids of native (1-133)human interleukin-3; or a
polypeptide having substantially the same structure and
substantially the same biological activity.

15

The present invention includes polypeptides of the
formula

| | | | | | |
|----|---|-----|-----|-----|-----------------------------|
| | 1 | | 5 | | 10 |
| | (Met) _m -Ala | Pro | Met | Thr | Gln Thr Thr Ser Leu Lys Thr |
| 20 | | 15 | | 20 | |
| | Ser Trp Val Asn Cys Ser Xaa Xaa Xaa Asp Glu Ile Ile | | | | |
| | 25 | | 30 | | 35 |
| | Xaa His Leu Lys Xaa Pro Pro Xaa Pro Xaa Leu Asp Xaa | | | | |
| | 40 | | 45 | | 50 |
| 25 | Xaa Asn Leu Asn Xaa Glu Asp Xaa Asp Ile Leu Xaa Glu | | | | |
| | | 55 | | 60 | |
| | Xaa Asn Leu Arg Xaa Xaa Asn Leu Xaa Xaa Phe Xaa Xaa | | | | |
| | 65 | | 70 | | 75 |
| | Ala Xaa Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa | | | | |
| 30 | | 80 | | 85 | |
| | Ile Leu Xaa Asn Leu Xaa Pro Cys Xaa Pro Xaa Xaa Thr | | | | |
| | 90 | | 95 | | 100 |
| | Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Ile Xaa Xaa Gly | | | | |
| | 105 | | 110 | | 115 |
| 35 | Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Xaa Phe Tyr Leu | | | | |
| | | 120 | | 125 | |

Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln Thr Thr Leu

130

Ser Leu Ala Ile Phe [SEQ ID NO:129]

- 5 wherein m is 0 or 1; Xaa at position 18 is Asn or Ile;
Xaa at position 19 is Met, Ala or Ile; Xaa at position
20 is Ile, Pro or Ile; Xaa at position 23 is Ile, Ala
or Leu; Xaa at position 25 is Thr or His; Xaa at
position 29 is Gln, Arg, Val or Ile; Xaa at position
10 32 is Leu, Ala, Asn or Arg; Xaa at position 34 is Leu
or Ser; Xaa at position 37 is Phe, Pro, or Ser; Xaa at
position 38 is Asn or Ala; Xaa at position 42 is Gly,
Ala, Ser, Asp or Asn; Xaa at position 45 is Gln, Val,
or Met; Xaa at position 46 is Asp or Ser; Xaa at
15 position 49 is Met, Ile, Leu or Asp; Xaa at position
50 is Glu or Asp; Xaa at position 51 is Asn Arg or
Ser; Xaa at position 55 is Arg, Leu, or Thr; Xaa at
position 56 is Pro or Ser; Xaa at position 59 is Glu
or Leu; Xaa at position 60 is Ala or Ser; Xaa at
20 position 62 is Asn, Val or Pro; Xaa at position 63 is
Arg or His; Xaa at position 65 is Val or Ser; Xaa at
position 67 is Ser, Asn, His or Gln; Xaa at position
69 is Gln or Glu; Xaa at position 73 is Ala or Gly;
Xaa at position 76 is Ser, Ala or Pro; Xaa at position
25 79 is Lys, Arg or Ser; Xaa at position 82 is Leu, Glu,
Val or Trp; Xaa at position 85 is Leu or Val; Xaa at
position 87 is Leu, Ser, Tyr; Xaa at position 88 is
Ala or Trp; Xaa at position 91 is Ala or Pro; Xaa at
position 93 is Pro or Ser; Xaa at position 95 is His
or Thr; Xaa at position 98 is His, Ile, or Thr; Xaa at
30 position 100 is Lys or Arg; Xaa at position 101 is
Asp, Ala or Met; Xaa at position 105 is Asn or Glu;
Xaa at position 109 is Arg, Glu or Leu; Xaa at
position 112 is Thr or Gln; Xaa at position 116 is
35 Lys, Val, Trp or Ser; Xaa at position 117 is Thr or
Ser; Xaa at position 120 is Asn, Gln, or His; Xaa at

position 123 is Ala or Glu; with the proviso that from four to twenty-six of the amino acids designated by Xaa are different from the corresponding amino acids of native human interleukin-3; or a polypeptide having
 5 substantially the same structure and substantially the same biological activity.

Preferred polypeptides of the present invention are those of the formula

| | | | |
|----|---|-------------------------------------|-----|
| | 1 | .5 | 10 |
| 10 | (Met _m -Ala _n) _p -Asn | Cys Ser Xaa Xaa Xaa Asp Glu Xaa Ile | |
| | 15 | 20 | |
| | Xaa His Leu Lys Xaa Pro Pro Xaa Pro Xaa Leu Asp Xaa | | |
| | 25 | 30 | 35 |
| | Xaa Asn Leu Asn Xaa Glu Asp Xaa Xaa Ile Leu Xaa Glu | | |
| 15 | 40 | 45 | |
| | Xaa Asn Leu Arg Xaa Xaa Asn Leu Xaa Xaa Phe Xaa Xaa | | |
| | 50 | 55 | 60 |
| | Ala Xaa Lys Xaa Leu Xaa Asn Ala Ser Xaa Ile Glu Xaa | | |
| | 65 | 70 | 75 |
| 20 | Ile Leu Xaa Asn Xaa Xaa Pro Cys Xaa Pro Xaa Ala Thr | | |
| | 80 | 85 | |
| | Ala Xaa Pro Xaa Arg Xaa Pro Ile Xaa Ile Xaa Xaa Gly | | |
| | 90 | 95 | 100 |
| | Asp Trp Xaa Glu Phe Arg Xaa Lys Leu Xaa Phe Tyr Leu | | |
| 25 | 105 | 110 | |
| | Xaa Xaa Leu Glu Xaa Ala Gln Xaa Gln Gln [SEQ ID | | |
| | NO:130] | | |

wherein m is 0 or 1; n is 0 or 1; p is 0 or 1; Xaa at
 30 position 4 is Asn or Ile; Xaa at position 5 is Met, Ala or Ile; Xaa at position 6 is Ile, Pro or Leu; Xaa at position 9 is Ile, Ala or Leu; Xaa at position 11 is Thr or His; Xaa at position 15 is Gln, Arg, Val or Ile; Xaa at position 18 is Leu, Ala, Asn or Arg; Xaa
 35 at position 20 is Leu or Ser; Xaa at position 23 is Phe, Pro, or Ser; Xaa at position 24 is Asn or Ala;

Xaa at position 28 is Gly, Ala, Ser, Asp or Asn; Xaa at position 31 is Gln, Val, or Met; Xaa at position 32 is Asp or Ser; Xaa at position 35 is Met, Ile or Asp; Xaa at position 36 is Glu or Asp; Xaa at position 37
5 is Asn, Arg or Ser; Xaa at position 41 is Arg, Leu, or Thr; Xaa at position 42 is Pro or Ser; Xaa at position 45 is Glu or Leu; Xaa at position 46 is Ala or Ser; Xaa at position 48 is Asn, Val or Pro; Xaa at position 49 is Arg or His; Xaa at position 51 is Val or Ser;
10 Xaa at position 53 is Ser, Asn, His or Gln; Xaa at position 55 is Gln or Glu; Xaa at position 59 is Ala or Gly; Xaa at position 62 is Ser, Ala or Pro; Xaa at position 65 is Lys, Arg or Ser; Xaa at position 67 is Leu, Glu, or Val; Xaa at position 68 is Leu, Glu, Val
15 or Trp; Xaa at position 71 is Leu or Val; Xaa at position 73 is Leu, Ser or Tyr; Xaa at position 74 is Ala or Trp; Xaa at position 77 is Ala or Pro; Xaa at position 79 is Pro or Ser; Xaa at position 81 is His or Thr; Xaa at position 84 is His, Ile, or Thr; Xaa at
20 position 86 is Lys or Arg; Xaa at position 87 is Asp, Ala or Met; Xaa at position 91 is Asn or Glu; Xaa at position 95 is Arg, Glu, Leu; Xaa at position 98 Thr or Gln; Xaa at position 102 is Lys, Val, Trp or Ser; Xaa at position 103 is Thr or Ser; Xaa at position 106
25 is Asn, Gln, or His; Xaa at position 109 is Ala or Glu; with the proviso that from four to twenty-six of the amino acids designated by Xaa are different from the corresponding amino acids of native (15-125)human interleukin-3; or a polypeptide having substantially
30 the same structure and substantially the same biological activity.

"Mutant amino acid sequence," "mutant protein" or "mutant polypeptide" refers to a polypeptide having an amino acid sequence which varies from a native
35 sequence or is encoded by a nucleotide sequence intentionally made variant from a native sequence.

"Mutant protein," "variant protein" or "mutein" means a protein comprising a mutant amino acid sequence and includes polypeptides which differ from the amino acid sequence of native hIL-3 due to amino acid deletions, substitutions, or both. "Native sequence" refers to an amino acid or nucleic acid sequence which is identical to a wild-type or native form of a gene or protein.

Human IL-3 can be characterized by its ability to stimulate colony formation by human hematopoietic progenitor cells. The colonies formed include erythroid, granulocyte, megakaryocyte, granulocytic macrophages and mixtures thereof. Human IL-3 has demonstrated an ability to restore bone marrow function and peripheral blood cell populations to therapeutically beneficial levels in studies performed initially in primates and subsequently in humans (Gillio, A. P., et al. (1990); Ganser, A, et al. (1990); Falk, S., et al. (1991). Additional activities of hIL-3 include the ability to stimulate leukocyte migration and chemotaxis; the ability to prime human leukocytes to produce high levels of inflammatory mediators like leukotrienes and histamine; the ability to induce cell surface expression of molecules needed for leukocyte adhesion; and the ability to trigger dermal inflammatory responses and fever. Many or all of these biological activities of hIL-3 involve signal transduction and high affinity receptor binding. Mutant polypeptides of the present invention may exhibit useful properties such as having similar or greater biological activity when compared to native hIL-3 or by having improved half-life or decreased adverse side effects, or a combination of these properties. They may also be useful as antagonists. hIL-3 mutant polypeptides which have little or no activity when compared to

native hIL-3 may still be useful as antagonists, as antigens for the production of antibodies for use in immunology or immunotherapy, as genetic probes or as intermediates used to construct other useful hIL-3
5 muteins. Since hIL-3 functions by binding to its receptor(s) and triggering second messages resulting in competent signal transduction, hIL-3 muteins of this invention may be useful in helping to determine which specific amino acid sequences are responsible
10 for these activities.

The novel hIL-3 mutant polypeptides of the present invention will preferably have at least one biological property of human IL-3 or of an IL-3-like growth factor and may have more than one IL-3-like
15 biological property, or an improved property, or a reduction in an undesirable biological property of human IL-3. Some mutant polypeptides of the present invention may also exhibit an improved side effect profile. For example, they may exhibit a decrease in
20 leukotriene release or histamine release when compared to native hIL-3 or (15-125) hIL-3. Such hIL-3 or hIL-3-like biological properties may include one or more of the following biological characteristics and in vivo and in vitro activities.

25 One such property is the support of the growth and differentiation of progenitor cells committed to erythroid, lymphoid, and myeloid lineages. For example, in a standard human bone marrow assay, an IL-3-like biological property is the stimulation of
30 granulocytic type colonies, megakaryocytic type colonies, monocyte/macrophage type colonies, and erythroid bursts. Other IL-3-like properties are the interaction with early multipotential stem cells, the sustaining of the growth of pluripotent precursor
35 cells, the ability to stimulate chronic myelogenous leukemia (CML) cell proliferation, the stimulation of

proliferation of mast cells, the ability to support the growth of various factor-dependent cell lines, and the ability to trigger immature bone marrow cell progenitors. Other biological properties of IL-3 have
5 been disclosed in the art. Human IL-3 also has some biological activities which may in some cases be undesirable, for example the ability to stimulate leukotriene release and the ability to stimulate increased histamine synthesis in spleen and bone
10 marrow cultures and in vivo.

Biological activity of hIL-3 and hIL-3 mutant proteins of the present invention is determined by DNA synthesis by human acute myelogenous leukemia cells (AML). The factor-dependent cell line AML 193 was
15 adapted for use in testing biological activity.

One object of the present invention is to provide hIL-3 muteins and hIL-3 deletion muteins with four or more amino acid substitutions in the polypeptide sequence which have similar or improved biological
20 activity in relation to native hIL-3 or native (15-125)hIL-3.

The present invention includes mutant polypeptides comprising minimally amino acids residues 15 to 118 of hIL-3 with or without additional amino
25 acid extensions to the N-terminus and/or C-terminus which further contain four or more amino acid substitutions in the amino acid sequence of the polypeptide. It has been found that the (15-125)hIL-3 mutant is more soluble than is hIL-3 when expressed in
30 the cytoplasm of E. coli, and the protein is secreted to the periplasm in E. coli at higher levels compared to native hIL-3.

When expressed in the E. coli cytoplasm, the above-mentioned mutant hIL-3 polypeptides of the
35 present invention may also be constructed with Met-Ala- at the N-terminus so that upon expression the Met

is cleaved off leaving Ala at the N-terminus. These mutant hIL-3 polypeptides may also be expressed in E. coli by fusing a signal peptide to the N-terminus. This signal peptide is cleaved from the polypeptide as
5 part of the secretion process. Secretion in E. coli can be used to obtain the correct amino acid at the N-terminus (e.g., Asn¹⁵ in the (15-125) hIL-3 polypeptide) due to the precise nature of the signal peptidase. This is in contrast to the heterogeneity
10 often observed at the N-terminus of proteins expressed in the cytoplasm in E. coli.

The hIL-3 mutant polypeptides of the present invention may have hIL-3 or hIL-3-like activity. For example, they may possess one or more of the
15 biological activities of native hIL-3 and may be useful in stimulating the production of hematopoietic cells by human or primate progenitor cells. The hIL-3 muteins of the present invention and pharmaceutical compositions containing them may be useful in the
20 treatment of conditions in which hematopoietic cell populations have been reduced or destroyed due to disease or to treatments such as radiation or chemotherapy.

hIL-3 muteins of the present invention may also
25 be useful as antagonists which block the hIL-3 receptor by binding specifically to it and preventing binding of the agonist.

One potential advantage of the (15-125) hIL-3 muteins of the present invention, particularly those
30 which retain activity similar to or better than that of native hIL-3, is that it may be possible to use a smaller amount of the biologically active mutein to produce the desired therapeutic effect. This may make it possible to reduce the number of treatments
35 necessary to produce the desired therapeutic effect. The use of smaller amounts may also reduce the

possibility of any potential antigenic effects or other possible undesirable side effects. For example, if a desired therapeutic effect can be achieved with a smaller amount of polypeptide it may be possible to
5 reduce or eliminate side effects associated with the administration of native IL-3 such as the stimulation of leukotriene and/or histamine release. The hIL-3 muteins of the present invention may also be useful in the activation of stem cells or progenitors which have
10 low receptor numbers. Pharmaceutical compositions containing (15-125) hIL-3 muteins of the present invention can be administered parenterally, intravenously, or subcutaneously.

As another aspect of the present invention, there
15 is provided a novel method for producing the novel family of human IL-3 muteins. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a vector containing a DNA sequence coding for expression of a
20 novel hIL-3 mutant polypeptide. Suitable cells or cell lines may be bacterial cells. For example, the various strains of E. coli are well-known as host cells in the field of biotechnology. Examples of such strains include E. coli strains JM101 [Yanish-Perron, et al. (1985)] and MON105 [Obukowicz, et al. (1992)].
25 Various strains of B. subtilis may also be employed in this method. Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the
30 present invention.

Also suitable for use in the present invention are mammalian cells, such as Chinese hamster ovary cells (CHO). General methods for expression of foreign genes in mammalian cells are reviewed in:
35 Kaufman, R. J. (1987) High level production of proteins in mammalian cells, in Genetic Engineering,

Principles and Methods, Vol. 9, J. K. Setlow, editor, Plenum Press, New York. An expression vector is constructed in which a strong promoter capable of functioning in mammalian cells drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant. For example, plasmids such as pcDNA I/Neo, pRc/RSV, and pRc/CMV (obtained from Invitrogen Corp., San Diego, California) can be used. The eukaryotic secretion signal peptide coding region can be from the hIL-3 gene itself or it can be from another secreted mammalian protein (Bayne, M. L. et al. (1987) Proc. Natl. Acad. Sci. USA 84, 2638-2642). After construction of the vector containing the hIL-3 variant gene, the vector DNA is transfected into mammalian cells. Such cells can be, for example, the COS7, HeLa, BHK, CHO, or mouse L lines. The cells can be cultured, for example, in DMEM media (JRH Scientific). The hIL-3 variant secreted into the media can be recovered by standard biochemical approaches following transient expression 24 - 72 hours after transfection of the cells or after establishment of stable cell lines following selection for neomycin resistance. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al., U.S. Pat. No. 4,419,446. Another suitable mammalian cell line is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298

(Plenum Press 1986) and references cited therein. In addition, general methods for expression of foreign genes in insect cells using Baculovirus vectors are described in: Summers, M. D. and Smith, G. E. (1987) -
5 A manual of methods for Baculovirus vectors and insect cell culture procedures, Texas Agricultural Experiment Station Bulletin No. 1555. An expression vector is constructed comprising a Baculovirus transfer vector, in which a strong Baculovirus promoter (such as the
10 polyhedron promoter) drives transcription of a eukaryotic secretion signal peptide coding region, which is translationally fused to the coding region for the hIL-3 variant polypeptide. For example, the plasmid pVL1392 (obtained from Invitrogen Corp., San
15 Diego, California) can be used. After construction of the vector carrying the hIL-3 variant gene, two micrograms of this DNA is cotransfected with one microgram of Baculovirus DNA (see Summers & Smith, 1987) into insect cells, strain SF9. Pure recombinant
20 Baculovirus carrying the hIL-3 variant is used to infect cells cultured, for example, in Excell 401 serum-free medium (JRH Biosciences, Lenexa, Kansas). The hIL-3 variant secreted into the medium can be recovered by standard biochemical approaches.
25 Another aspect of the present invention provides plasmid DNA vectors for use in the method of expression of these novel hIL-3 muteins. These vectors contain the novel DNA sequences described above which code for the novel polypeptides of the
30 invention. Appropriate vectors which can transform microorganisms capable of expressing the hIL-3 muteins include expression vectors comprising nucleotide sequences coding for the hIL-3 muteins joined to transcriptional and translational regulatory sequences
35 which are selected according to the host cells used.

Vectors incorporating modified sequences as

described above are included in the present invention and are useful in the production of the hIL-3 mutant polypeptides. The vector employed in the method also contains selected regulatory sequences in operative
5 association with the DNA coding sequences of the invention and capable of directing the replication and expression thereof in selected host cells.

Additional details may be found in co-filed United States Patent Application Attorney docket
10 number 2713/1, which is hereby incorporated by reference in its entirety.

All references, patents or applications cited herein are incorporated by reference in their
15 entirety.

The present invention also includes the construction and expression of (15-125)human interleukin-3 muteins having four or more amino acid substitutions in secretion vectors that optimize
20 accumulation of correctly folded, active polypeptide. While many heterologous proteins have been secreted in E. coli there is still a great deal of unpredictability and limited success (Stader and Silhavy 1990). Full-length hIL-3 is such a protein,
25 where attempts to secrete the protein in E. coli resulted in low secretion levels. Secretion of the variant (15-125) hIL-3 mutant polypeptides of the present invention as a fusion with a signal peptide such as LamB results in correctly folded protein that
30 can be removed from the periplasm of E. coli by osmotic shock fractionation. This property of the variant (15-125) hIL-3 muteins allows for the direct and rapid screening for bioactivity of the secreted material in the crude osmotic shock fraction, which is
35 a significant advantage. Furthermore, it provides a means of using the (15-125)hIL-3 muteins to conduct

structure activity relationship (SAR) studies of the hIL-3 molecule. A further advantage of secretion of (15-125) hIL-3 muteins fused to the LamB signal peptide is that the secreted polypeptide has the correct N-terminal amino acid (Asn) due to the precise nature of the cleavage of the signal peptide by signal peptidase, as part of the secretion process.

The (15-125)hIL-3 muteins of the present invention may include hIL-3 polypeptides having Met-, Ala- or Met-Ala- attached to the N-terminus. When the muteins are expressed in the cytoplasm of E. coli, polypeptides with and without Met attached to the N-terminus are obtained. The methionine can in some cases be removed by methionine aminopeptidase.

Amino terminal sequences of hIL-3 muteins made in E. coli were determined using the method described by Hunkapillar et al., (1983). It was found that hIL-3 proteins made in E. coli from genes encoding Met-(15-125)hIL-3 were isolated as Met-(15-125) hIL-3.

Proteins produced from genes encoding Met-Ala-(15-125) hIL-3 were produced as Ala-(15-125) hIL-3. The N-termini of proteins made in the cytoplasm of E. coli are affected by posttranslational processing by methionine aminopeptidase (Ben-Bassat et al., 1987) and possibly by other peptidases.

One method of creating the preferred hIL-3 (15-125) mutant genes is cassette mutagenesis [Wells, et al. (1985)] in which a portion of the coding sequence of hIL-3 in a plasmid is replaced with synthetic oligonucleotides that encode the desired amino acid substitutions in a portion of the gene between two restriction sites. In a similar manner amino acid substitutions could be made in the full-length hIL-3 gene, or genes encoding variants of hIL-3 in which from 1 to 14 amino acids have been deleted from the N-terminus and/or from 1 to 15 amino acids have been

deleted from the C-terminus. When properly assembled these oligonucleotides would encode hIL-3 variants with the desired amino acid substitutions and/or deletions from the N-terminus and/or C-terminus.

- 5 These and other mutations could be created by those skilled in the art by other mutagenesis methods including; oligonucleotide-directed mutagenesis [Zoller and Smith (1982, 1983, 1984), Smith (1985), Kunkel (1985), Taylor, et al. (1985), Deng and
10 Nickoloff (1992)] or polymerase chain reaction (PCR) techniques [Saiki, (1985)].

- Pairs of complementary synthetic oligonucleotides encoding portions of the amino terminus of the hIL-3 gene can be made and annealed to each other. Such
15 pairs would have protruding ends compatible with ligation to NcoI at one end. The NcoI site would include the codon for the initiator methionine. At the other end of oligonucleotide pairs, the protruding (or blunt) ends would be compatible with a restriction
20 site that occurs within the coding sequence of the hIL-3 gene. The DNA sequence of the oligonucleotide would encode sequence for amino acids of hIL-3 with the exception of those substituted and/or deleted from the sequence.

- 25 The NcoI enzyme and the other restriction enzymes chosen should have recognition sites that occur only once in the DNA of the plasmid chosen. Plasmid DNA can be treated with the chosen restriction endonucleases then ligated to the annealed
30 oligonucleotides. The ligated mixtures can be used to transform competent JM101 cells to resistance to an appropriate antibiotic. Single colonies can be picked and the plasmid DNA examined by restriction analysis and/or DNA sequencing to identify plasmids with mutant
35 hIL-3 genes.

One example of a restriction enzyme which cleaves

within the coding sequence of the hIL-3 gene is ClaI whose recognition site is at codons 20 and 21. The use of ClaI to cleave the sequence of hIL-3 requires that the plasmid DNA be isolated from an E. coli strain that fails to methylate adenines in the DNA at GATC recognition sites. This is because the recognition site for ClaI, ATCGAT, occurs within the sequence GATCGAT which occurs at codons 19, 20 and 21 in the hIL-3 gene. The A in the GATC sequence is methylated in most E. coli host cells. This methylation prevents ClaI from cleaving at that particular sequence. An example of a strain that does not methylate adenines is GM48.

Interpretation of activity of single amino acid mutants in IL-3 (15-125)

As illustrated in Tables 6 and 9, there are certain positions in the IL-3 (15-125) molecule which are intolerant of substitutions, in that most or all substitutions at these positions resulted in a considerable decrease in bioactivity. There are two likely classes of such "down-mutations": mutations that affect overall protein structure, and mutations that interfere directly with the interaction between the IL-3 molecule and its receptor. Mutations affecting the three-dimensional structure of the protein will generally lie in the interior of the protein, while mutations affecting receptor binding will generally lie on the surface of the protein.

Although the three-dimensional structure of IL-3 is unknown, there are simple algorithms which can aid in the prediction of the structure. One such algorithm is the use of "helical wheels" (Kaiser, E.T. & Kezdy, F.J., Science, 223:249-255 (1984)). In this method, the presence of alpha helical protein structures can be predicted by virtue of their amphipathic nature.

Helices in globular proteins commonly have an exposed hydrophilic side and a buried hydrophobic side. As a broad generalization, in globular proteins, hydrophobic residues are present in the interior of the protein, and hydrophilic residues are present on the surface. By displaying the amino acid sequence of a protein on such a "helical wheel" it is possible to derive a model for which amino acids in alpha helices are exposed and which are buried in the core of the protein. Such an analysis of the IL-3 (15-125) molecule predicts that the following helical residues are buried in the core:

M19, I20, I23, I24, L27, L58, F61, A64, L68, A71, I74, I77, L78, L81, W104, F107, L111, Y114, L115, L118.

In addition, cysteine residues at positions 16 and 84 are linked by a disulfide bond, which is important for the overall structure or "folding" of the protein. Finally, mutations which result in a major disruption of the protein structure may be expressed at low level in the secretion system used in our study, for a variety of reasons: either because the mis-folded protein is poorly recognized by the secretion machinery of the cell; because mis-folding of the protein results in aggregation, and hence the protein cannot be readily extracted from the cells; or because the mis-folded protein is more susceptible to degradation by cellular proteases. Hence, a block in secretion may indicate which positions in the IL-3 molecule which are important for maintenance of correct protein structure.

In order to retain the activity of a variant of IL-3, it is necessary to retain both the structural

integrity of the protein, and retain the specific residues important for receptor contact. Hence it is possible to define specific amino acid residues in IL-3 (15-125) which must be retained in order to preserve biological activity.

Residues predicted to be important for interaction with the receptor: D21, E22, E43, D44, L48, R54, R94, D103, K110, F113.

10

Residues predicted to be structurally important: C16, L58, F61, A64, I74, L78, L81, C84, P86, P92, P96, F107, L111, L115, L118.

15

The hIL-3 muteins of the present invention may be useful in the treatment of diseases characterized by a decreased levels of either myeloid, erythroid, lymphoid, or megakaryocyte cells of the hematopoietic system or combinations thereof. In addition, they may be used to activate mature myeloid and/or lymphoid cells. Among conditions susceptible to treatment with the polypeptides of the present invention is leukopenia, a reduction in the number of circulating leukocytes (white cells) in the peripheral blood. Leukopenia may be induced by exposure to certain viruses or to radiation. It is often a side effect of various forms of cancer therapy, e.g., exposure to chemotherapeutic drugs and of infection or hemorrhage. Therapeutic treatment of leukopenia with these hIL-3 mutant polypeptides of the present invention may avoid undesirable side effects caused by treatment with presently available drugs.

The hIL-3 muteins of the present invention may be useful in the treatment of neutropenia and, for example, in the treatment of such conditions as aplastic anemia, cyclic neutropenia, idiopathic neutropenia, Chediak-Higashi syndrome, systemic lupus erythematosus (SLE), leukemia, myelodysplastic syndrome and myelofibrosis.

Many drugs may cause bone marrow suppression or hematopoietic deficiencies. Examples of such drugs are AZT, DDI, alkylating agents and anti-metabolites used in chemotherapy, antibiotics such as chloramphenicol, penicillin and sulfa drugs, phenothiazones, tranquilizers such as meprobamate, and diuretics. The hIL-3 muteins of the present invention may be useful in preventing or treating the bone marrow suppression or hematopoietic deficiencies which often occur in patients treated with these drugs.

Hematopoietic deficiencies may also occur as a

result of viral, microbial or parasitic infections and as a result of treatment for renal disease or renal failure, e.g., dialysis. The hIL-3 muteins of the present invention may be useful in treating such

5 hematopoietic deficiency.

The treatment of hematopoietic deficiency may include administration of the hIL-3 mutein of a pharmaceutical composition containing the hIL-3 mutein to a patient. The hIL-3 muteins of the present

10 invention may also be useful for the activation and amplification of hematopoietic precursor cells by treating these cells in vitro with the muteins of the present invention prior to injecting the cells into a patient.

15 Various immunodeficiencies e.g., in T and/or B lymphocytes, or immune disorders, e.g., rheumatoid arthritis, may also be beneficially affected by treatment with the hIL-3 mutant polypeptides of the present invention. Immunodeficiencies may be the

20 result of viral infections e.g. HTLV I, HTLV II, HTLV III, severe exposure to radiation, cancer therapy or the result of other medical treatment. The hIL-3 mutant polypeptides of the present invention may also be employed, alone or in combination with other

25 hematopoietins, in the treatment of other blood cell deficiencies, including thrombocytopenia (platelet deficiency), or anemia. Other uses for these novel polypeptides are in the treatment of patients recovering from bone marrow transplants in vivo and ex

30 vivo, and in the development of monoclonal and polyclonal antibodies generated by standard methods for diagnostic or therapeutic use.

Other aspects of the present invention are methods and therapeutic compositions for treating the

35 conditions referred to above. Such compositions comprise a therapeutically effective amount of one or

more of the hIL-3 muteins of the present invention in a mixture with a pharmaceutically acceptable carrier. This composition can be administered either parenterally, intravenously or subcutaneously. When
5 administered, the therapeutic composition for use in this invention is preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such a parenterally acceptable protein solution, having due regard to pH, isotonicity,
10 stability and the like, is within the skill of the art.

The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician considering
15 various factors which modify the action of drugs, e.g. the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, a daily regimen may be in the range of 0.2 - 150 µg/kg
20 of non-glycosylated IL-3 protein per kilogram of body weight. This dosage regimen is referenced to a standard level of biological activity which recognizes that native IL-3 generally possesses an EC₅₀ at or about 10 piconMolar to 100 piconMolar in the AML
25 proliferation assay described herein. Therefore, dosages would be adjusted relative to the activity of a given mutein vs. the activity of native (reference) IL-3 and it would not be unreasonable to note that dosage regimens may include doses as low as 0.1
30 microgram and as high as 1 milligram per kilogram of body weight per day. In addition, there may exist specific circumstances where dosages of IL-3 mutein would be adjusted higher or lower than the range of 10 - 200 micrograms per kilogram of body weight. These
35 include co-administration with other CSF or growth factors; co-administration with chemotherapeutic drugs

and/or radiation; the use of glycosylated IL-3 mutein; and various patient-related issues mentioned earlier in this section. As indicated above, the therapeutic method and compositions may also include co-

5 administration with other human factors. A non-exclusive list of other appropriate hematopoietins, CSFs and interleukins for simultaneous or serial co-administration with the polypeptides of the present invention includes GM-CSF, CSF-1, G-CSF, Meg-CSF, M-

10 CSF, erythropoietin (EPO), IL-1, IL-4, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, LIF, B-cell growth factor, B-cell differentiation factor and eosinophil differentiation factor, stem cell factor (SCF) also known as steel factor or c-kit ligand, or combinations

15 thereof. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by periodic assessment of the hematological profile, e.g., differential cell count

20 and the like.

Materials and methods for hIL-3 Mutein Expression in E. coli

Unless noted otherwise, all specialty chemicals were obtained from Sigma Co., (St. Louis, MO).

25 Restriction endonucleases, T4 poly-nucleotides kinase, E. coli DNA polymerase I large fragment (Klenow) and T4 DNA ligase were obtained from New England Biolabs (Beverly, Massachusetts).

Escherichia coli strains

30 Strain JM101: delta (pro lac), supE, thi, F'(traD36, rpoAB, lacI-Q, lacZdeltaM15) (Messing, 1979). This strain can be obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, accession number 33876.

35 MON 105 (W3110 rpoH358) is a derivative of W3110 (Bachmann, 1972) and has been assigned ATCC accession

number 55204. Strain GM48: *dam-3*, *dcm-6*, *gal*, *ara*, *lac*, *thr*, *leu*, *tonA*, *tsx* (Marinus, 1973) was used to make plasmid DNA that is not methylated at the sequence GATC.

5 Genes and plasmids

The gene used for hIL-3 production in *E. coli* was obtained from British Biotechnology Incorporated, Cambridge, England, catalogue number BBG14. This gene is carried on a pUC based plasmid designated pP0518.

10 The plasmids used for production of hIL-3 in *E. coli* contain genetic elements whose use has been described (Olins et al., 1988; Olins and Rangwala, 1990). The replicon used is that of pBR327 (Covarrubias, et al., 1981) which is maintained at a
15 copy number of about 100 in the cell (Soberon et al., 1980). A gene encoding the beta-lactamase protein is present on the plasmids. This protein confers ampicillin resistance on the cell. This resistance serves as a selectable phenotype for the presence of
20 the plasmid in the cell.

For cytoplasmic expression vectors the transcription promoter was derived from the *recA* gene of *E. coli* (Sancar et al., 1980). This promoter, designated *precA*, includes the RNA polymerase binding
25 site and the *lexA* repressor binding site (the operator). This segment of DNA provides high level transcription that is regulated even when the *recA* promoter is on a plasmid with the pBR327 origin of replication (Olins et al., 1988) incorporated herein
30 by reference.

In secretion expression plasmids the transcription promoter was derived from the *ara B*, *A*, and *D* genes of *E. coli* (Greenfield et al., 1978). This promoter is designated *pAraBAD* and is contained
35 on a 323 base pair *SacII*, *BglIII* restriction fragment. The *LamB* secretion leader (Wong et al., 1988, Clement

et al., 1981) was fused to the N-terminus of the hIL-3 gene at the recognition sequence for the enzyme *NcoI* (5'CCATGG3'). The hIL-3 genes used were engineered to have a *HindIII* recognition site (5'AAGCTT3') following the coding sequence of the gene.

These hIL-3 variants were expressed as a fusion with the LamB signal peptide shown in Figure 8, operatively joined to the araBAD promoter (Greenfield, 1978) and the gl0-L ribosome binding site (Olins et al. 1988). The processed form was selectively released from the periplasm by osmotic shock as a correctly folded and fully active molecule. Secretion of (15-125) hIL-3 was further optimized by using low inducer (arabinose) concentration and by growth at 30°C. These conditions resulted in lower accumulation levels of unprocessed LamB signal peptide (15-125) hIL-3 fusion, maximal accumulation levels of processed (15-125) hIL-3 and selective release of (15-125) hIL-3 by osmotic shock fractionation. The use of a tightly regulated promoter such as araBAD from which the transcription level and hence the expression level can be modulated allowed for the optimization of secretion of (15-125) hIL-3.

The ribosome binding site used is that from gene 10 of phage T7 (Olins et al., 1988). This is encoded in a 100 base pair (bp) fragment placed adjacent to precA. In the plasmids used herein, the recognition sequence for the enzyme *NcoI* (CCATGG) follows the gl0-L. It is at this *NcoI* site that the hIL-3 genes are joined to the plasmid. It is expected that the nucleotide sequence at this junction will be recognized in mRNA as a functional start site for translation (Olins et al., 1988). The hIL-3 genes used were engineered to have a *HindIII* recognition site (AAGCTT) downstream from the coding sequence of the gene. At this *HindIII* site is a 514 base pair

RsaI fragment containing the origin of replication of the single stranded phage f1 (Dente et al., 1983; Olins, et al., 1990) both incorporated herein by reference. A plasmid containing these elements is pMON2341. Another plasmid containing these elements is pMON5847 which has been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 under the accession number ATCC 68912.

10 Synthesis of Oligonucleotides

Oligonucleotides were synthesized on Nucleotide Synthesizer model 380A or 380B from Applied Biosystems, Inc. (Foster City, California). Oligonucleotides were purified by polyacrylamide gel electrophoresis at concentrations from 12 - 20% (19:1 crosslinked) in 0.5 x Tris borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA) followed by passage through a Nensorb column obtained from New England Nuclear (Boston, Massachusetts) using a PREP Automated Sample Processor obtained from DuPont, Co. (Wilmington, Delaware).

Quantitation of synthetic oligonucleotides

Synthetic oligonucleotides were resuspended in water and quantitated by reading the absorbance at 260nm on a Beckman DU40 Spectrophotometer (Irvine, California) using a one centimeter by one millimeter quartz cuvette (Maniatis, 1982). The concentration was determined using an extinction coefficient of 1×10^4 (Voet et al., 1963; Mahler and Cordes, 1966). The oligonucleotides were then diluted to a desired concentration.

Quantitation of synthetic DNA fragments can also be achieved by adding 10 to 100 picomoles of DNA to a solution containing kinase buffer (25 mM Tris pH 8.0, 10 mM $MgCl_2$, 10 mM DTT and 2 mM spermidine). To the reaction mix is added ATP to 20 micromolar, ATP

radiolabeled at the gamma phosphate (5000-10,000 dpm/pmol) and 5 units of T4 polynucleotide kinase. Radiolabelled material is obtained from New England Nuclear (Boston, Massachusetts). The 10 microliter mixture is incubated at 37°C for one hour. A 1 microliter aliquot of the mixture was chromatographed on DEAE paper (Whatman) in 0.3 M ammonium bicarbonate. The counts that remained at the origin were used to determine the concentration of the synthetic DNA.

Recombinant DNA methods

Isolation of plasmid DNA from E. coli cultures was performed as described (Birnboim and Doly, 1979). Some DNAs were purified by Magic™ columns, available from Promega (Madison, Wisconsin).

Purified plasmid DNA was treated with restriction endonucleases according to manufacturer's instructions. Analysis of the DNA fragments produced by treatment with restriction enzymes was done by agarose or polyacrylamide gel electrophoresis. Agarose (DNA grade from Fisher, Pittsburgh PA.) was used at a concentration of 1.0% in a Tris-acetate running buffer (0.04 M Tris-acetate, 0.001M EDTA). Polyacrylamide (BioRad, Richmond CA.) was used at a concentration of 6% (19:1 crosslinked) in 0.5 X Tris-borate buffer (0.045 M Tris, 0.045 M boric acid, 1.25 mM EDTA), hereafter referred to as PAGE.

DNA polymerase I, large fragment, Klenow enzyme was used according to manufacturers instructions to catalyze the addition of mononucleotides from 5' to 3' of DNA fragments which had been treated with restriction enzymes that leave protruding ends. The reactions were incubated at 65°C for 10 minutes to heat inactivate the Klenow enzyme.

The synthetic oligonucleotides were made without 5' or 3' terminal phosphates. In cases where such

oligonucleotides were ligated end to end, the oligonucleotides were treated at a concentration of 10 picomoles per microliter with T4 polynucleotide kinase in the following buffer: 25 mM Tris, pH 8.0, 10 mM MgCl₂, 10 mM dithiothreitol, 2 mM spermidine, 1 mM rATP. After incubation for 30 minutes at 37°C, the samples were incubated at 65°C for five minutes to heat inactivate the kinase.

Synthetic gene assembly

10 The (15-125) hIL-3 gene was divided into four regions separated by five convenient restriction sites. In each of the four regions synthetic oligonucleotides were designed so that they would anneal in complementary pairs, with protruding single stranded ends, and when the pairs were properly
15 assembled would result in a DNA sequence that encoded a portion of the hIL-3 gene. Amino acid substitutions in the hIL-3 gene were made by designing the oligonucleotides to encode the desired substitutions.

20 The complementary oligonucleotides were annealed at concentration of 1 picomole per microliter in ligation buffer plus 50mM NaCl. The samples were heated in a 100 ml beaker of boiling water and permitted to cool slowly to room temperature. One picomole of each of
25 the annealed pairs of oligonucleotides were ligated with approximately 0.2 picomoles of plasmid DNA, digested with the appropriate restriction enzymes, in ligation buffer (25 mM Tris pH 8.0, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP, 2mM spermidine) with T4 DNA
30 ligase obtained from New England Biolabs (Beverly, Massachusetts) in a total volume of 20 µl at room temperature overnight.

 DNA fragments were isolated from agarose gels by intercepting the restriction fragments on DEAE
35 membranes from Schleicher and Schuell (Keene, New Hampshire) and eluting the DNA in 10 mM Tris, 1 mM

EDTA, 1 M NaCl at 55°C for 1 hour, according to manufacturer's directions. The solutions containing the DNA fragment were concentrated and desalted by using Centricon 30 concentrators from Amicon (W.R. Grace, Beverly MA.) according to the manufacturer's directions. Ligations were performed at 15°C overnight, except as noted, in ligation buffer.

Polymerase Chain Reaction

10 Polymerase Chain Reaction (hereafter referred to as PCR) techniques (Saiki, 1985) used the reagent kit and thermal cycler from Perkin-Elmer Cetus (Norwalk, CT.). PCR is based on a thermostable DNA polymerase from *Thermus aquaticus*. The PCR technique is a DNA
15 amplification method that mimics the natural DNA replication process in that the number of DNA molecules doubles after each cycle, in a way similar to in vivo replication. The DNA polymerase mediated extension is in a 5' to 3' direction. The term
20 "primer" as used herein refers to an oligonucleotide sequence that provides an end to which the DNA polymerase can add nucleotides that are complementary to a nucleotide sequence. The latter nucleotide sequence is referred to as the "template", to which
25 the primers are annealed. The amplified PCR product is defined as the region comprised between the 5' ends of the extension primers. Since the primers have defined sequences, the product will have discrete ends, corresponding to the primer sequences. The primer
30 extension reaction was carried out using 20 picomoles (pmoles) of each of the oligonucleotides and 1 picogram of template plasmid DNA for 35 cycles (1 cycle is defined as 94 degrees C for one minute, 50 degrees C for two minutes and 72 degrees for three
35 minutes.). The reaction mixture was extracted with an equal volume of phenol/chloroform (50% phenol and 50%

chloroform, volume to volume) to remove proteins. The aqueous phase, containing the amplified DNA, and solvent phase were separated by centrifugation for 5 minutes in a microcentrifuge (Model 5414 Eppendorf Inc, Fremont CA.). To precipitate the amplified DNA the aqueous phase was removed and transferred to a fresh tube to which was added 1/10 volume of 3M NaOAc (pH 5.2) and 2.5 volumes of ethanol (100% stored at minus 20 degrees C). The solution was mixed and placed on dry ice for 20 minutes. The DNA was pelleted by centrifugation for 10 minutes in a microcentrifuge and the solution was removed from the pellet. The DNA pellet was washed with 70% ethanol, ethanol removed and dried in a speedvac concentrator (Savant, Farmingdale, New York). The pellet was resuspended in 25 microliters of TE (20mM Tris-HCl pH 7.9, 1mM EDTA). Alternatively the DNA was precipitated by adding equal volume of 4M NH₄OAc and one volume of isopropanol [Treco et al., (1988)]. The solution was mixed and incubated at room temperature for 10 minutes and centrifuged. These conditions selectively precipitate DNA fragments larger than ~ 20 bases and were used to remove oligonucleotide primers. One quarter of the reaction was digested with restriction enzymes [Higuchi, (1989)] and on completion heated to 70 degrees C to inactivate the enzymes.

Recovery of recombinant plasmids from ligation mixes

E. coli JM101 cells were made competent to take up DNA. Typically, 20 to 100 ml of cells were grown in LB medium to a density of approximately 150 Klett units and then collected by centrifugation. The cells were resuspended in one half culture volume of 50 mM CaCl₂ and held at 4°C for one hour. The cells were again collected by centrifugation and resuspended in one tenth culture volume of 50 mM CaCl₂. DNA was

added to a 150 microliter volume of these cells, and the samples were held at 4°C for 30 minutes. The samples were shifted to 42°C for one minute, one milliliter of LB was added, and the samples were
5 shaken at 37°C for one hour. Cells from these samples were spread on plates containing ampicillin to select for transformants. The plates were incubated overnight at 37°C. Single colonies were picked, grown in LB supplemented with ampicillin overnight at 37°C
10 with shaking. From these cultures DNA was isolated for restriction analysis.

Culture medium

LB medium (Maniatis et al., 1982) was used for
15 growth of cells for DNA isolation. M9 minimal medium supplemented with 1.0% casamino acids, acid hydrolyzed casein, Difco (Detroit, Michigan) was used for cultures in which recombinant hIL-3 was produced. The ingredients in the M9 medium were as follows:
20 3g/liter KH_2PO_4 , 6g/l Na_2HPO_4 , 0.5 g/l NaCl , 1 g/l NH_4Cl , 1.2 mM MgSO_4 , 0.025 mM CaCl_2 , 0.2% glucose (0.2% glycerol with the AraBAD promoter), 1% casamino acids, 0.1 ml/l trace minerals (per liter 108 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 4.0 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 7.0 $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$, 7.0 g
25 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 8.0 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2.0 g H_3BO_3 , 5.0 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 100 ml concentrated HCl). Bacto agar was used for solid media and ampicillin was added to both liquid and solid LB media at 200 micrograms per milliliter.

30

DNA sequence analysis

The nucleotide sequencing of plasmid DNA was determined using a Genesis 2000 sequencer obtained from DuPont (Wilmington, Delaware) according to the
35 methods of Prober et al. (1987) and Sanger et al. (1977). Some DNA sequences were performed using

Sequenase™ polymerase (U.S. Biochemicals, Cleveland, Ohio) according to manufacturer's directions.

Production of recombinant hIL-3 muteins in E. coli
5 with vectors employing the recA promoter

E. coli strains harboring the plasmids of interest were grown at 37°C in M9 plus casamino acids medium with shaking in a Gyrotory water bath Model G76 from New Brunswick Scientific (Edison, New Jersey).
10 Growth was monitored with a Klett Summerson meter (green 54 filter), Klett Mfg. Co. (New York, New York). At a Klett value of approximately 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining
15 culture, nalidixic acid (10mg/ml) in 0.1 N NaOH was added to a final concentration of 50 µg/ml. The cultures were shaken at 37°C for three to four hours after addition of nalidixic acid. A high degree of aeration was maintained throughout the bacterial
20 growth in order to achieve maximal production of the desired gene product. The cells were examined under a light microscope for the presence of refractile bodies (RBs). One milliliter aliquots of the culture were removed for analysis of protein content.

25 Production of recombinant hIL-3 proteins from the pAraBAD promoter in E. coli

E. coli strains harboring the plasmids of interest were grown at 30°C with shaking in M9 medium plus casamino acids and glycerol. Growth was
30 monitored with a Klett Summerson colorimeter, using a green 54 filter. At a Klett value of about 150, an aliquot of the culture (usually one milliliter) was removed for protein analysis. To the remaining culture, 20% arabinose was added to a final
35 concentration of 0.05%. The cultures were shaken at 30°C for three to four hours after addition of

arabinose. A high degree of aeration was maintained throughout the bacterial growth in order to achieve maximal production of the desired gene product. One milliliter aliquots of the culture were removed for
5 analysis of protein content.

Secretion and osmotic shock

Three hour post induction samples were fractionated by osmotic shock [Neu and Heppel (1965)]. The optical density (Klett value) of the cultures was
10 determined and 1 ml of cells were centrifuged in a Sigma microcentrifuge (West Germany) model 202MK in 1.5 mls snap top microcentrifuge tubes for 5 minutes at 10,000 rpm. The cell pellet was resuspended very gently by pipeting in a room temperature sucrose
15 solution (20% sucrose w/v, 30mM Tris-HCl pH7.5, 1mM EDTA), using 1 μ l/1 Klett unit. Following a 10 minute incubation at room temperature, the cells were centrifuged for 5 minutes at 10,000 rpm. The sucrose fraction was carefully removed from the cell pellet.
20 The cell pellet was then resuspended very gently by pipeting in ice cold distilled water, using 1 μ l/1 Klett unit. Following a 10 minute incubation on ice, the cells were centrifuged for 5 minutes at 12,000 rpm. The water fraction was carefully removed. Equal
25 volumes of the sucrose and water fractions were pooled and aliquoted to provide samples for activity screening.

Analysis of protein content of E. coli cultures producing hIL-3 mutant polypeptides

30 Bacterial cells from cultures treated as described above were collected from the medium by centrifugation. Aliquots of these cells were resuspended in SDS loading buffer (4X: 6 g SDS, 10 ml beta-mercaptoethanol, 25 ml upper Tris gel stock (0.5
35 M Tris HCl pH 6.8, 0.4% SDS) brought to 50 ml with glycerol, 0.2% bromophenol blue was added) at a

concentration of one microliter per Klett unit. These samples were incubated at 85°C for five minutes and vortexed. Five or ten microliter aliquots of these samples were loaded on 15% polyacrylamide gels prepared according to the method of Laemmli (1970). Protein bands were visualized by staining the gels with a solution of acetic acid, methanol and water at 5:1:5 ratio (volume to volume) to which Coomassie blue had been added to a final concentration of 1%. After staining, the gels were washed in the same solution without the Coomassie blue and then washed with a solution of 7% acetic acid, 5% methanol. Gels were dried on a gel drier Model SE1160 obtained from Hoeffer (San Francisco, California). The amount of stained protein was measured using a densitometer obtained from Joyce-Loebl (Gateshead, England). The values obtained were a measure of the amount of the stained hIL-3 protein compared to the total of the stained protein of the bacterial cells.

20 Western blot analysis of hIL-3 muteins made in E. coli

In some E. coli cultures producing hIL-3, the level of accumulation of the hIL-3 protein is lower than 5% of total bacterial protein. To detect hIL-3 produced at this level, Western blot analysis was used. Proteins from cultures induced with nalidixic acid or arabinose were run on polyacrylamide gels as described above except that volumes of sample loaded were adjusted to produce appropriate signals. After electrophoresis, the proteins were electroblotted to APT paper, Transa-bind, Schleicher and Schuell (Keene, New Hampshire) according to the method of Renart et al. (1979). Antisera used to probe these blots had been raised in rabbits, using peptides of the sequence of amino acids 20 to 41 and 94 to 118 of hIL-3 as the immunogens. The presence of bound antibody was detected with Staphylococcal protein A radiolabeled

with ^{125}I , obtained from New England Nuclear (Boston, Massachusetts).

Fractionation of *E. coli* cells producing hIL-3 proteins in the cytoplasm

5 Cells from *E. coli* cultures harboring plasmids that produce hIL-3 muteins were induced with nalidixic acid. After three hours, the hIL-3 muteins accumulated in refractile bodies. The first step in purification of the hIL-3 muteins was to sonicate
10 cells. Aliquots of the culture were resuspended from cell pellets in sonication buffer: 10 mM Tris, pH 8.0, 1 mM EDTA, 50 mM NaCl and 0.1 mM PMSF. These resuspended cells were subjected to several repeated sonication bursts using the microtip from a Sonicator
15 cell disrupter, Model W-375 obtained from Heat Systems-Ultrasonics Inc. (Farmingdale, New York). The extent of sonication was monitored by examining the homogenates under a light microscope. When nearly all of the cells had been broken, the homogenates were
20 fractionated by centrifugation. The pellets, which contain most of the refractile bodies, are highly enriched for hIL-3 muteins.

Methods: Extraction, Refolding and Purification of Interleukin-3 (IL-3) Muteins Expressed as Refractile Bodies in *E. coli*.

Extraction of refractile bodies (RB's):

For each gram of RB's (and typically one gram is obtained from a 300 ml *E. coli* culture), 5 ml of a solution containing 6M guanidine hydrochloride
30 (GnHCl), 50 mM 2-N-cyclohexylaminoethanesulfonic acid (CHES) pH 9.5 and 20 mM dithiothreitol (DTT) was added. The RB's were extracted with a Bio-Homogenizer for 15-30 seconds and gently rocked for 2 hours at 5 degrees centigrade (5°C) to allow the protein to
35 completely reduce and denature.

Refolding of the IL-3 muteins

The protein solution was transferred to dialysis tubing (1000 molecular weight cut-off) and dialyzed against at least 100 volumes of 4M GnHCl - 50 mM CHES pH 8.0. The dialysis was continued overnight at 5°C while gently stirring. Subsequently dialysis was continued against at least 100 volumes of 2M GnHCl - 50 mM CHES pH 8.0 and dialyzed overnight at 5°C while gently stirring.

Purification of the IL-3 muteins

The protein solution was removed from the dialysis tubing and acidified by the addition of 40% acetonitrile (CH₃CN) - 0.2% trifluoroacetic acid (TFA) to a final concentration of 20% CH₃CN - 0.1% TFA. This was centrifuged (16,000 x g for 5 minutes) to clarify and the supernatant was loaded onto a Vydac C-18 reversed phase column (10x250 mm) available from Vydac (Hesperia, California) previously equilibrated in 20% CH₃CN - 0.1% TFA. The column was eluted with a linear gradient (0.2% CH₃CN/minute) between 40 - 50% CH₃CN - 0.1% TFA at a flow rate of 3 ml/minute while collecting 1.5 ml fractions. The fractions were analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and the appropriate fractions pooled. The pooled material was dried by lyophilization or in a Speed Vac concentrator. The dry powder was reconstituted with 10 mM ammonium bicarbonate pH 7.5, centrifuged (16,000 x g for 5 minutes) to clarify and assayed for protein concentration by the method of Bradford (1976) with bovine serum albumin as the standard. Such protein can be further analyzed by additional techniques such as, SDS-PAGE, electrospray mass spectrometry, reverse phase HPLC, capillary zone electrophoresis, amino acid composition analysis, and ELISA (enzyme-linked immunosorbent assay).

hIL-3 SANDWICH ELISA

IL-3 protein concentrations can be determined using a sandwich ELISA based on an affinity purified polyclonal goat anti-rhIL-3. Microtiter plates (Dynatech Immulon II) were coated with 150 μ l goat-
5 anti-rhIL-3 at a concentration of approximately 1 μ g/ml in 100 mM NaHCO₃, pH 8.2. Plates were incubated overnight at room temperature in a chamber maintaining 100% humidity. Wells were emptied and the remaining reactive sites on the plate were blocked with 200 μ l
10 of solution containing 10 mM PBS, 3% BSA and 0.05% Tween 20, pH 7.4 for 1 hour at 37° C and 100% humidity. Wells were emptied and washed 4X with 150 mM NaCl containing 0.05% Tween 20 (wash buffer). Each well then received 150 μ l of dilution buffer (10 mM
15 PBS containing 0.1% BSA, 0.01% Tween 20, pH 7.4), containing rhIL-3 standard, control, sample or dilution buffer alone. A standard curve was prepared with concentrations ranging from 0.125 ng/ml to 5 ng/ml using a stock solution of rhIL-3 (concentration
20 determined by amino acid composition analysis). Plates were incubated 2.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 μ l of an optimal dilution (as determined in a
25 checkerboard assay format) of goat anti-rhIL-3 conjugated to horseradish peroxidase. Plates were incubated 1.5 hours at 37° C and 100% humidity. Wells were emptied and each plate was washed 4X with wash buffer. Each well then received 150 μ l of ABTS
30 substrate solution (Kirkegaard and Perry). Plates were incubated at room temperature until the color of the standard wells containing 5 ng/ml rhIL-3 had developed enough to yield an absorbance between 0.5-1.0 when read at a test wavelength of 410 nm and a
35 reference wavelength of 570 nm on a Dynatech microtiter plate reader. Concentrations of

immunoreactive rhIL-3 in unknown samples were calculated from the standard curve using software supplied with the plate reader.

5 AML Proliferation Assay for Bioactive Human
Interleukin-3

The factor-dependent cell line AML 193 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This cell line, established
10 from a patient with acute myelogenous leukemia, is a growth factor dependent cell line which displayed enhanced growth in GM/CSF supplemented medium (Lange, B., et al., (1987); Valtieri, M., et al., (1987). The ability of AML 193 cells to proliferate
15 in the presence of human IL-3 has also been documented. (Santoli, D., et al., (1987)). A cell line variant was used, AML 193 1.3, which was adapted for long term growth in IL-3 by washing out the growth factors and starving the cytokine dependent AML 193
20 cells for growth factors for 24 hours. The cells were then replated at 1×10^5 cells/well in a 24 well plate in media containing 100 U/ml IL-3. It took approximately 2 months for the cells to grow rapidly in IL-3. These cells were maintained as AML 193 1.3
25 thereafter by supplementing tissue culture medium (see below) with human IL-3.

AML 193 1.3 cells were washed 6 times in cold Hanks balanced salt solution (HBSS, Gibco, Grand
Island, NY) by centrifuging cell suspensions at $250 \times$
30 g for 10 minutes followed by decantation of supernatant. Pelleted cells were resuspended in HBSS and the procedure was repeated until six wash cycles were completed. Cells washed six times by this procedure were resuspended in tissue culture medium at
35 a density ranging from 2×10^5 to 5×10^5 viable cells/ml. This medium was prepared by supplementing

Iscove's modified Dulbecco's Medium (IMDM, Hazleton, Lenexa, KS) with albumin, transferrin, lipids and 2-mercaptoethanol. Bovine albumin (Boehringer-Mannheim, Indianapolis, IN) was added at 500 µg/ml; human

5 transferrin (Boehringer-Mannheim, Indianapolis, IN) was added at 100 µg/ml; soybean lipid (Boehringer-Mannheim, Indianapolis, IN) was added at 50 µg/ml; and 2-mercaptoethanol (Sigma, St. Louis, MO) was added at 5×10^{-5} M.

10 Serial dilutions of human interleukin-3 or human interleukin-3 variant protein (hIL-3 mutein) were made in triplicate series in tissue culture medium supplemented as stated above in 96 well Costar 3596 tissue culture plates. Each well contained 50 µl of

15 medium containing interleukin-3 or interleukin-3 variant protein once serial dilutions were completed. Control wells contained tissue culture medium alone (negative control). AML 193 1.3 cell suspensions prepared as above were added to each well by pipetting

20 50 µl (2.5×10^4 cells) into each well. Tissue culture plates were incubated at 37°C with 5% CO₂ in humidified air for 3 days. On day 3, 0.5 µCi ³H-thymidine (2 Ci/mM, New England Nuclear, Boston, MA) was added in 50 µl of tissue culture medium. Cultures

25 were incubated at 37°C with 5% CO₂ in humidified air for 18-24 hours. Cellular DNA was harvested onto glass filter mats (Pharmacia LKB, Gaithersburg, MD) using a TOMTEC cell harvester (TOMTEC, Orange, CT) which utilized a water wash cycle followed by a 70%

30 ethanol wash cycle. Filter mats were allowed to air dry and then placed into sample bags to which scintillation fluid (Scintiverse II, Fisher Scientific, St. Louis, MO or BetaPlate Scintillation Fluid, Pharmacia LKB, Gaithersburg, MD) was added.

35 Beta emissions of samples from individual tissue culture wells were counted in a LKB Betaplate model

1205 scintillation counter (Pharmacia LKB, Gaithersburg, MD) and data was expressed as counts per minute of ^3H -thymidine incorporated into cells from each tissue culture well. Activity of each human interleukin-3 preparation or human interleukin-3 variant preparation was quantitated by measuring cell proliferation (^3H -thymidine incorporation) induced by graded concentrations of interleukin-3 or interleukin-3 variant. Typically, concentration ranges from 0.05 pM - 10^5 pM are quantitated in these assays. Activity is determined by measuring the dose of interleukin-3 or interleukin-3 variant which provides 50% of maximal proliferation [$\text{EC}_{50} = 0.5 \times (\text{maximum average counts per minute of } ^3\text{H}\text{-thymidine incorporated per well among triplicate cultures of all concentrations of interleukin-3 tested - background proliferation measured by } ^3\text{H}\text{-thymidine incorporation observed in triplicate cultures lacking interleukin-3}]$. This EC_{50} value is also equivalent to 1 unit of bioactivity. Every assay was performed with native interleukin-3 as a reference standard so that relative activity levels could be assigned.

Relative biological activities of IL-3 muteins of the present invention are shown in Table 1. The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC_{50} of (1-133) hIL-3 by the EC_{50} of the mutant. The Relative Biological Activity may be the average of replicate assays.

TABLE 1

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

| | | |
|----|-----------|-------------|
| 35 | Relative* | |
| | Plasmid | Polypeptide |

Biological

| Code | | Structure | |
|----------|-----------|----------------|-----|
| Activity | Reference | (1-133)hIL-3 | |
| <u>1</u> | | | |
| 5 | pMON13298 | SEQ ID NO. 82 | 3 |
| | pMON13299 | SEQ ID NO. 83 | 2 |
| | pMON13300 | SEQ ID NO. 84 | 3 |
| | pMON13301 | SEQ ID NO. 85 | 2 |
| 10 | pMON13302 | SEQ ID NO. 86 | 1.2 |
| | pMON13303 | SEQ ID NO. 87 | 0.6 |
| | pMON13287 | SEQ ID NO. 88 | 26 |
| | pMON13288 | SEQ ID NO. 89 | 24 |
| | pMON13289 | SEQ ID NO. 90 | 13 |
| 15 | pMON13290 | SEQ ID NO. 91 | 20 |
| | pMON13292 | SEQ ID NO. 92 | 6 |
| | pMON13294 | SEQ ID NO. 93 | 3 |
| | pMON13295 | SEQ ID NO. 94 | 3 |
| | pMON13312 | SEQ ID NO. 95 | 4 |
| 20 | pMON13313 | SEQ ID NO. 96 | 8 |
| | pMON13285 | SEQ ID NO. 259 | 32 |
| | pMON13286 | SEQ ID NO. 260 | 8 |
| | pMON13325 | SEQ ID NO. 261 | 8 |
| | pMON13326 | SEQ ID NO. 262 | 25 |
| 25 | pMON13330 | SEQ ID NO. 263 | 19 |
| | pMON13329 | SEQ ID NO. 406 | 10 |
| | pMON13364 | SEQ ID NO. 117 | 13 |

TABLE 1 (cont'd)

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

| 5 Relative* | | | |
|-------------|-----------|----------------|------|
| Plasmid | | Polypeptide | |
| Biological | | | |
| Code | Structure | Activity | |
| 10 | pMON13475 | SEQ ID NO. 280 | 7 |
| | pMON13366 | SEQ ID NO. 281 | 38 |
| | pMON13367 | SEQ ID NO. 282 | 36 |
| | pMON13368 | SEQ ID NO. 278 | 1.6 |
| | pMON13369 | SEQ ID NO. 283 | 10 |
| 15 | pMON13370 | SEQ ID NO. 284 | 6 |
| | pMON13373 | SEQ ID NO. 285 | 12 |
| | pMON13374 | SEQ ID NO. 286 | 6 |
| | pMON13375 | SEQ ID NO. 287 | 14 |
| | pMON13376 | SEQ ID NO. 288 | 0.4 |
| 20 | pMON13377 | SEQ ID NO. 289 | 0.4 |
| | pMON13379 | SEQ ID NO. 291 | 0.9 |
| | pMON13380 | SEQ ID NO. 279 | 0.05 |
| | pMON13381 | SEQ ID NO. 293 | 10 |
| | pMON13382 | SEQ ID NO. 313 | 38 |
| 25 | pMON13383 | SEQ ID NO. 294 | 0.5 |
| | pMON13384 | SEQ ID NO. 295 | 0.25 |
| | pMON13385 | SEQ ID NO. 292 | 1 |
| | pMON13387 | SEQ ID NO. 308 | 32 |
| | pMON13388 | SEQ ID NO. 296 | 23 |
| 30 | pMON13389 | SEQ ID NO. 297 | 10 |
| | pMON13391 | SEQ ID NO. 298 | 30 |
| | pMON13392 | SEQ ID NO. 299 | 17 |
| | pMON13393 | SEQ ID NO. 300 | 32 |
| | pMON13394 | SEQ ID NO. 301 | 20 |
| 35 | pMON13395 | SEQ ID NO. 302 | 11 |
| | pMON13396 | SEQ ID NO. 303 | 20 |
| | pMON13397 | SEQ ID NO. 304 | 16 |
| | pMON13398 | SEQ ID NO. 305 | 36 |
| | pMON13399 | SEQ ID NO. 306 | 18 |
| 40 | pMON13404 | SEQ ID NO. 307 | 1.3 |
| | pMON13417 | SEQ ID NO. 310 | 24 |
| | pMON13420 | SEQ ID NO. 311 | 19 |
| | pMON13421 | SEQ ID NO. 312 | 0.5 |
| | pMON13432 | SEQ ID NO. 313 | 10 |
| 45 | pMON13400 | SEQ ID NO. 317 | 0.09 |
| | pMON13402 | SEQ ID NO. 318 | 20 |
| | pMON13403 | SEQ ID NO. 321 | 0.03 |
| | pMON13405 | SEQ ID NO. 267 | 9 |

TABLE 1 (cont'd)

BIOLOGICAL ACTIVITY OF IL-3 MUTEINS

| 5 | Relative* | | |
|----|------------|----------------|----------|
| | Plasmid | Polypeptide | |
| | Biological | | |
| | Code | Structure | Activity |
| 10 | pMON13406 | SEQ ID NO. 264 | 5 |
| | pMON13407 | SEQ ID NO. 266 | 16 |
| | pMON13408 | SEQ ID NO. 269 | 7 |
| | pMON13409 | SEQ ID NO. 270 | 15 |
| 15 | pMON13410 | SEQ ID NO. 271 | 0.4 |
| | pMON13411 | SEQ ID NO. 322 | 1.2 |
| | pMON13412 | SEQ ID NO. 323 | 0.5 |
| | pMON13413 | SEQ ID NO. 324 | 0.6 |
| | pMON13414 | SEQ ID NO. 265 | 4 |
| 20 | pMON13415 | SEQ ID NO. 268 | 4 |
| | pMON13418 | SEQ ID NO. 326 | 0.5 |
| | pMON13419 | SEQ ID NO. 325 | 0.015 |
| | pMON13422 | SEQ ID NO. 272 | 0.4 |
| 25 | pMON13423 | SEQ ID NO. 273 | 0.4 |
| | pMON13424 | SEQ ID NO. 274 | 3 |
| | pMON13425 | SEQ ID NO. 275 | 6 |
| | pMON13426 | SEQ ID NO. 276 | >0.0003 |
| 30 | pMON13429 | SEQ ID NO. 277 | >0.0002 |
| | pMON13440 | SEQ ID NO. 319 | 9 |
| | pMON13451 | SEQ ID NO. 320 | 0.1 |
| | pMON13459 | SEQ ID NO. 328 | 0.003 |
| 35 | pMON13416 | SEQ ID NO. 309 | 19.9 |
| | pMON13428 | SEQ ID NO. 327 | 0.008 |
| | pMON13467 | SEQ ID NO. 329 | 0.16 |
| 40 | pMON13446 | SEQ ID NO. 315 | 21.5 |
| | pMON13390 | SEQ ID NO. 316 | 20 |

* The Relative Biological Activity of IL-3 mutants is calculated by dividing the EC₅₀ of (1-133) hIL-3 by the EC₅₀ of the mutant.

The following assay is used to measure IL-3

mediated sulfidoleukotriene release from human mononuclear cells.

IL-3 mediated sulfidoleukotriene release from human mononuclear cells

Heparin-containing human blood was collected and layered onto an equal volume of Ficoll-Paque (Pharmacia # 17-0840-02) ready to use medium (density 1.077 g/ml.). The Ficoll was warmed to room temperature prior to use and clear 50 ml polystyrene tubes were utilized. The Ficoll gradient was spun at 300 x g for 30 minutes at room temperature using a H1000B rotor in a Sorvall RT6000B refrigerated centrifuge. The band containing the mononuclear cells was carefully removed, the volume adjusted to 50 mls with Dulbecco's phosphate-buffered saline (Gibco Laboratories cat. # 310-4040PK), spun at 400 x g for 10 minutes at 4°C and the supernatant was carefully removed. The cell pellet was washed twice with HA Buffer [20 mM Hepes (Sigma # H-3375), 125 mM NaCl (Fisher # S271-500), 5 mM KCl (Sigma # P-9541), 0.5 mM glucose (Sigma # G-5000), 0.025% Human Serum Albumin (Calbiochem # 126654) and spun at 300 x g, 10 min., 4°C. The cells were resuspended in HACM Buffer (HA buffer supplemented with 1 mM CaCl₂ (Fisher # C79-500) and 1 mM MgCl₂ (Fisher # M-33) at a concentration of 1 x 10⁶ cells/ml and 180 µl were transferred into each well of 96 well tissue culture plates. The cells were allowed to acclimate at 37°C for 15 minutes. The cells were primed by adding 10 µls of a 20 X stock of various concentrations of cytokine to each well (typically 100000, 20000, 4000, 800, 160, 32, 6.4, 1.28, 0 fM IL3). The cells were incubated for 15 minutes at 37°C. Sulfidoleukotriene release was activated by the addition of 10 µls of 20 X (1000 nM)

fmet-leu-phe (Calbiochem # 344252) final concentration 50nM FMLP and incubated for 10 minutes at 37°C. The plates were spun at 350 x g at 4°C for 20 minutes. The supernatants were removed and assayed for sulfidoleukotrienes using Cayman's Leukotriene C4 EIA kit (Cat. #420211) according to manufacturers' directions. Native (15-125)hIL-3 was run as a standard control in each assay.

Native hIL-3 possesses considerable inflammatory activity and has been shown to stimulate synthesis of the arachidonic acid metabolites LTC₄, LTD₄, and LTE₄; histamine synthesis and histamine release. Human clinical trials with native hIL-3 have documented inflammatory responses (Biesma, et al., BLOOD, 80:1141-1148 (1992) and Postmus, et al., J. CLIN. ONCOL., 10:1131-1140 (1992)). A recent study indicates that leukotrienes are involved in IL-3 actions in vivo and may contribute significantly to the biological effects of IL-3 treatment (Denzlinger, C., et al., BLOOD, 81:2466-2470 (1993))

Some muteins of the present invention may have an improved therapeutic profile as compared to native hIL-3 or (15-125)hIL-3. For example, some muteins of the present invention may have a similar or more potent growth factor activity relative to native hIL-3 or (15-125)hIL-3 without having a similar or corresponding increase in the stimulation of leukotriene or histamine. These muteins would be expected to have a more favorable therapeutic profile since the amount of polypeptide which needs to be given to achieve the desired growth factor activity (e. g. cell proliferation) would have a lesser leukotriene or histamine stimulating effect. In studies with native hIL-3, the stimulation of inflammatory factors has been an undesirable side

effect of the treatment. Reduction or elimination of the stimulation of mediators of inflammation would provide an advantage over the use of native hIL-3.

The pMON13288 polypeptide has demonstrated a more
5 potent growth factor activity relative to native hIL-3
in the AML 193 cell proliferation assay ($EC_{50} = 0.8 -$
3.8 pM for pMON13288 and $EC_{50} = 30.2$ pM for native
hIL-3) without demonstrating a corresponding increase
in the stimulation of leukotriene C₄ (LTC₄) production
10 and histamine release, i. e., it stimulated LTC₄
production and histamine release with a potency
similar to that of native hIL-3 while having an
improved ability to stimulate cell proliferation
compared to native hIL-3. Thus with the pMON13288
15 polypeptide it would be expected that one would be
able to produce a desired therapeutic response, e. g.,
cell proliferation, with less stimulation of the
undesirable inflammatory mediators.

Some muteins of the present invention have
20 antigenic profiles which differ from that of native
hIL-3. For example, in a competition ELISA with an
affinity purified polyclonal goat anti-hIL-3 antibody,
native hIL-3 significantly blocked the binding of
labeled hIL-3 to polyclonal anti-hIL-3 antibody
25 whereas the pMON13288 polypeptide failed to block the
binding of hIL-3 to anti-hIL-3 antibody.

Table 2 lists the sequences of some
oligonucleotides used in making the muteins of the
present invention.

30 Table 3 lists the amino acid sequence of native
(15-125)hIL-3 (Peptide #1) and the amino acid
sequences of some mutant polypeptides of the present
invention. The sequences are shown with the amino acid
numbering corresponding to that of native hIL-3 [FIG.
35 1].

Table 4 lists the nucleotide sequences of some

DNA sequences which encode mutant polypeptides of the present invention.

TABLE 2
OLIGONUCLEOTIDES

5

Oligo #1 Length: 000040

CATGGCTAAC TGCTCTATAA TGATCGATGA AATTATACAT [SEQ ID NO:15]

10

Oligo #2 Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATCAT TATAGAGCAG TTAGC [SEQ ID NO:16]

15

Oligo #3 Length: 000036

CACTTAAAGA GACCACCTGC ACCTTTGCTG GACCCG [SEQ ID NO:17]

Oligo #4 Length: 000036

GAGGTTGTTC GGGTCCAGCA AAGGTGCAGG TGGTCT [SEQ ID NO:18]

20

Oligo #5 Length: 000036

CACTTAAAGA GACCACCTAA CCCTTTGCTG GACCCG [SEQ ID NO:19]

Oligo #6 Length: 000036

25

GAGGTTGTTC GGGTCCAGCA AAGGGTTAGG TGGTCT [SEQ ID NO:20]

Oligo #7 Length: 000036

CACTTAAAGG TTCCACCTGC ACCTTTGCTG GACAGT [SEQ ID NO:21]

30

Oligo #8 Length: 000036

GAGGTTGTTA CTGTCCAGCA AAGGTGCAGG TGAAC [SEQ ID NO:22]

Oligo #9 Length: 000027

AACAACCTCA ATGCTGAAGA CGTTGAT [SEQ ID NO:23]

35

Oligo #10 Length: 000018

ATCAACGTCT TCAGCATT [SEQ ID NO:24]

Oligo #11 Length: 000027

AACAACCTCA ATTCTGAAGA CATGGAT [SEQ ID NO:25]

5

Oligo #12 Length: 000018

ATCCATGTCT TCAGAATT [SEQ ID NO:26]

Oligo #13 Length: 000022

10 CATGGGAACC ATATGTCAGG AT [SEQ ID NO:27]

Oligo #14 Length: 000018

ATCCTGACAT ATGGTTCC [SEQ ID NO:28]

15 Oligo #15 Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:29]

Oligo #16 Length: 000024

AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:30]

20

Oligo #17 Length: 000020

AATTCGAACC ATATGTCAGA [SEQ ID NO:31]

Oligo #18 Length: 000020

25 AGCTTCTGAC ATATGGTTTCG [SEQ ID NO:32]

Oligo #19 Length: 000022

ATCGAACCAT ATGTCAGATG CA [SEQ ID NO:33]

30 Oligo #20 Length: 000018

TCTGACATAT GGTTCGAT [SEQ ID NO:34]

Oligo #21 Length: 000036

ATCCTGATGG AACGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:35]

35

Oligo #22 Length: 000027

AAGTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:36]

Oligo #23 Length: 000036

ATCCTGATGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:37]

5

Oligo #24 Length: 000027

AGTTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:38]

Oligo #25 Length: 000024

10 CTCGCATTCG TAAGGGCTGT CAAG [SEQ ID NO:39]

Oligo #26 Length: 000024

CCTTACGAAT GCGAGCAGGT TTGG [SEQ ID NO:40]

15 Oligo #27 Length: 000024

GAGAGCTTCG TAAGGGCTGT CAAG [SEQ ID NO:41]

Oligo #28 Length: 000024

20 CCTTACGAAG CTCTCCAGGT TTGG [SEQ ID NO:42]

Oligo #29 Length: 000015

CACTTAGAAA ATGCA [SEQ ID NO:43]

25 Oligo #30 Length: 000020

TTTTCTAAGT GCTTGACAGC [SEQ ID NO:44]

Oligo #31 Length: 000015

AACTTAGAAA ATGCA [SEQ ID NO:45]

30

Oligo #32 Length: 000020

TTTTCTAAGT TCTTGACAGC [SEQ ID NO:46]

Oligo #33 Length: 000048

35 GGTGATTGGA TGTCGAGAGG GTGCGGCCGT GGCAGAGGGC AGACATGG
[SEQ ID NO:47]

Oligo #34 Length: 000048
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA CCATCAAG
[SEQ ID NO:48]

5

Oligo #35 Length: 000048
GATGATTGGA TGTCGAGAGG GTGCGGCCGT GGCAGAGGGC AGACATGG
[SEQ ID NO:49]

10

Oligo #36 Length: 000048
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA TCATCAAG
[SEQ ID NO:50]

15

Oligo #37 Length: 000018
TACGAGATTA CGAAGAAT [SEQ ID NO:51]

Oligo #38 Length: 000018
CGTAATCTCG TACCATGT [SEQ ID NO:52]

20

Oligo #39 Length: 000018
TTGGAGATTA CGAAGAAT [SEQ ID NO:53]

Oligo #40 Length: 000018
CGTAATCTCC AACCATGT [SEQ ID NO:54]

25

Oligo #41 Length: 000019
TGCCTCAATA CCTGATGCA [SEQ ID NO:55]

Oligo #42 Length: 000021
TCAGGTATTG AGGCAATTCT T [SEQ ID NO:56]

30

Oligo #43 Length: 000026
AATTCTTGCC AGTCACCTGC CTTGAT [SEQ ID NO:57]

35

Oligo #44 Length: 000016
GCAGGTGACT GGCAAG [SEQ ID NO:58]

- Oligo #45 Length: 000032
AATTCGGGGA AAAACTGACG TTCTATCTGG TT [SEQ ID NO:59]
- 5 Oligo #46 Length: 000037
CTCAAGGGAA ACCAGATAGA ACGTCAGTTT TTCCCGG [SEQ ID NO:60]
- Oligo #47 Length: 000032
ACCCTTGAGC ACGCGCAGGA ACAACAGTAA TA [SEQ ID NO:61]
- 10 Oligo #48 Length: 000027
AGCTTATTAC TGTGTTTCCT GCGCGTG [SEQ ID NO:62]
- Oligo #49 Length: 000032
15 ACCCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:63]
- Oligo #50 Length: 000027
AGCTTATTAC TGTGTTTCCT GCGCTTG [SEQ ID NO:64]
- 20 Oligo #51 Length: 000034
GCCGATACCGCGGCATACTCCCACCATTGAGAGA [SEQ ID NO:155]
- Oligo #52 Length: 000033
25 GCCGATAAGATCTAAAACGGGTATGGAGAAACA [SEQ ID NO:156]
- Oligo #53
ATAGTCTTCCCCAGATATCTAACGCTTGAG [SEQ ID NO:157]
- Oligo #54 Length: 24
30 CAATACCTGATGCGTTTTCTAAGT [SEQ ID NO:158]
- Oligo #55 Length: 33
GGTTTCGTTCCATCAGAATGTCCATGTCTTCAG [SEQ ID NO:159]
- 35 Oligo #165 NCOECRV1.REQ Length: 000040.
CATGGCTAAC TGCTCTAACA TGATCGATGA AATTATAACA [SEQ ID
NO:162]
- 40 Oligo #166 NCOECRV4.REQ Length: 000045

CTTTAAGTGT GTTATAATTT CATCGATCAT GTTAGAGCAG TTAGC [SEQ ID
NO:163]

5 Oligo #167 NCOECRV2.REQ Length: 000036

CACTTAAAGC AGCCACCTTT GCCTTGCTG GACTTC [SEQ ID NO:164]

Oligo #168 NCOECRV5.REQ Length: 000036

10 GAGGTTGTTG AAGTCCAGCA AAGGCAAAGG TGGCTG [SEQ ID NO:165]

Oligo #169 2D5M6SUP.REQ Length: 000027

15 AACCAACCTCA ATGACGAAGA CATGTCT [SEQ ID NO:166]

Oligo #170 2D5M6SLO.REQ Length: 000018

AGACATGTCT TCGTCATT [SEQ ID NO:167]

20 Oligo #15(A) Length: 000016

TGAACCATAT GTCAGG [SEQ ID NO:168]

Oligo #16(A) Length: 000024

25 AATTCCTGAC ATATGGTTCA TGCA [SEQ ID NO:169]

Oligo #B1 19ALA1.REQ Length: 000040

CATGGCAAAC TGCTCTATAG CTATCGATGA AATTATACAT [SEQ ID NO:170]

30 Oligo #B2 19ALA4.REQ Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATAGC TATAGAGCAG TTTGC [SEQ ID
NO:171]

35 Oligo #B3 19ILE1.REQ Length: 000040

CATGGCAAAC TGCTCTATAA TCATCGATGA AATTATACAT [SEQ ID NO:172]

40 Oligo #B4 19ILE4.REQ Length: 000045

CTTTAAGTGA TGTATAATTT CATCGATGAT TATAGAGCAG TTTGC [SEQ ID
NO:173]

45 Oligo #B5 49ASP1.REQ Length: 000036

ATCCTGGACG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:174]

Oligo #B6 49ASP4.REQ Length: 000027

50 AGTTCGAAGG TTTCGTTTCGT CCAGGAT [SEQ ID NO:175]

Oligo #B7 49ILE1.REQ Length: 000036

ATCCTGATCG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:176]

5 Oligo #B8 49ILE4.REQ Length: 000027
AGTTCGAAGG TTTCGTTCCA TCAGGAT [SEQ ID NO:177]

Oligo #B9 49LEU1.REQ Length: 000036

10 ATCCTGCTGG AACGAAACCT TCGAACTCCA AACCTG [SEQ ID NO:178]

Oligo #B10 49LEU4.REQ Length: 000027
AGTTCGAAGG TTTCGTTCCA GCAGGAT [SEQ ID NO:179]

15 Oligo #B11 42S45V3.REQ Length: 000027
AACAACTCA ATTCTGAAGA CGTTGAT [SEQ ID NO:180]

20 Oligo #B12 42S45V6.REQ Length: 000018
ATCAACGTCT TCAGAATT [SEQ ID NO:181]

Oligo #B13 18I23A5H.REQ Length: 000051

25 CGCGCCATGG CTAAGTCTC TATAATGATC GATGAAGCAA TACATCACTTA
[SEQ ID NO:182]

Oligo #B14 2341HIN3.REQ Length: 000018

30 CGCGTCGATA AGCTTATT [SEQ ID NO:183]

Oligo #B15 2341NCO.REQ Length: 000018

35 GGAGATATAT CCATGGCT [SEQ ID NO:184]

Oligo #B16 2A5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCAGC ATTGAGGTTG TT [SEQ ID

40 NO:185]

Oligo #B17 2A5V6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGAAA CGTCTTCAGC ATTGAGGTTG TT [SEQ ID

45 NO:186]

Oligo #B18 2D5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCGTC ATTGAGGTTG TT [SEQ ID

50 NO:187]

Oligo #B19 2D5V6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGAAA CGTCTTCGTC ATTGAGGTTG TT [SEQ ID

55 NO:188]

Oligo #B20 2S5M6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGACA TGTCTTCAGA ATTGAGGTTG TT [SEQ ID

60 NO:189]

Oligo #B21 2S5V6S0D.REQ Length: 000042
TCGGTCCATC AGAATAGAAA CGTCTTCAGA ATTGAGGTTG TT [SEQ ID
NO:190]

5 Oligo #B22 100ARG3.REQ Length: 000048
CTGCCCTCTG CCACGGCCGC ACCCTCTCGA CATCCAATCA TCATCCGT [SEQ
ID NO:191]

10 Oligo #B23 100ARG8.REQ Length: 000026
AATTCTTGCC AGTCACCTGC ACGGAT [SEQ ID NO:192]

15 Oligo #B24 101MET4.REQ Length: 000016
ATGGGTGACT GGCAAG [SEQ ID NO:193]

20 Oligo #B25 10R01M8.REQ Length: 000026
AATTCTTGCC AGTCACCCAT ACGGAT [SEQ ID NO:194]

Oligo #B26 23ALA1.REQ Length: 000040

25 CATGGCTAAC TGCTCTATTA TGATCGATGA AGCAATACAT [SEQ ID NO:195]

Oligo #B27 23ALA4.REQ Length: 000045
CTTTAAGTGA TGTATTGCTT CATCGATCAT AATAGAGCAG TTAGC [SEQ ID
NO:196]

30 Oligo #B28 29V2R4S2.REQ Length: 000036
CACTTAAAGG TACCACCTCG CCCTTCCCTG GACCCG [SEQ ID NO:197]

35 Oligo #B29 29V2R4S5.REQ Length: 000036
GAGGTTGTTC GGGTCCAGGG AAGGGCGAGG TGGTAC [SEQ ID NO:198]

40 Oligo #B30 34SER2.REQ Length: 000036
CACTTAAAGA GACCACCTGC ACCTTCCCTG GACCCG [SEQ ID NO:199]

45 Oligo #B31 34SER5.REQ Length: 000036
GAGGTTGTTC GGGTCCAGGG AAGGTGCAGG TGGTCT [SEQ ID NO:200]

Oligo #B32 42D45M3.REQ Length: 000027

50 AACAACTCA ATGACGAAGA CATGGAT [SEQ ID NO:201]

Oligo #B33 42D45M6.REQ Length: 000018
ATCCATGTCT TCGTCATT [SEQ ID NO:202]

55 Oligo #B34 42D45V3.REQ Length: 000027
AACAACTCA ATGACGAAGA CGTCGAT [SEQ ID NO:203]

60 Oligo #B35 42D45V6.REQ Length: 000018

ATCGACGTCT TCGTCATT [SEQ ID NO:204]
Oligo #B36 42D5M6S3.REQ Length: 000027
5 AACAACTCA ATGACGAAGA CATGTCT [SEQ ID NO:205]
Oligo #B37 42D5M6S6.REQ Length: 000018
AGACATGTCT TCGTCATT [SEQ ID NO:206]
10 Oligo #B38 42D5V6S3.REQ Length: 000027
AACAACTCA ATGACGAAGA CGTCTCT [SEQ ID NO:207]
15 Oligo #B39 42D5V6S6.REQ Length: 000018
AGAGACGTCT TCGTCATT [SEQ ID NO:208]
Oligo #B40 50ASP1.REQ Length: 000036
20 ATCCTGATGG ACCGAAACCT TCGACTTCCA AACCTG [SEQ ID NO:209]
Oligo #B41 50ASP4.REQ Length: 000027
25 AAGTCGAAGG TTTCGGTCCA TCAGGAT [SEQ ID NO:210]
Oligo #B42 50D56S1.REQ Length: 000036
ATCCTGATGG ACCGAAACCT TCGACTTAGC AACCTG [SEQ ID NO:211]
30 Oligo #B43 56SER5.REQ Length: 000024
CCTTACGAAG CTCTCCAGGT TGCT [SEQ ID NO:212]
35 Oligo #B44 82TRP2.REQ Length: 000018
CGTAATCTCT GGCCATGT [SEQ ID NO:213]
Oligo #B45 82TRP6.REQ Length: 000018
40 CCAGAGATTA CGAAGAAT [SEQ ID NO:214]

Oligo #B46 9E12Q6W1.REQ Length: 000032
 AATTCGGGA AAAACTGCAA TTCTATCTGT GG [SEQ ID NO:215]

5 Oligo #B47 9E12Q6W3.REQ Length: 000037
 CTCAAGGGTC CACAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:216]

10 Oligo #B48 9E12Q6V1.REQ Length: 000032
 AATTCGGGA AAAACTGCAA TTCTATCTGG TT [SEQ ID NO:217]

 Oligo #B49 9E12Q6V3.REQ Length: 000037
 15 CTCAAGGGTA ACCAGATAGA ATTGCAGTTT TTCCCGG [SEQ ID NO:218]

 Oligo #B50 S09E16V1.REQ Length: 000023
 AATTCGGGA AAAACTGACG TTC [SEQ ID NO:219]

20 Oligo #B51 S09E16V3.REQ Length: 000028
 AACCAGATAG AACGTCAGTT TTTCCCGG [SEQ ID NO:220]

25 Oligo #B52 S116VD31.REQ Length: 000023
 TATCTGGTTA CCCTTGAGTA ATA [SEQ ID NO:221]

30 Oligo #B53 SECR1D33.REQ Length: 000018
 AGCTTATTAC TTCAAGGGT [SEQ ID NO:222]

 Oligo #B54 S9E2Q6V1.REQ Length: 000023
 35 AATTCGGGA AAAACTGCAA TTC [SEQ ID NO:223]

 Oligo #B55 S9E2Q6V3.REQ Length: 000028
 AACCAGATAG AATTGCAGTT TTTCCCGG [SEQ ID NO:224]

40 Oligo #B56 Ent338.Lo Length: 61
 CGATCATTAT AGAGCAGTTA GCCTTGTCAT CGTCGTCCTT GTAATCAGTT
 TCTGGATATG C [SEQ ID NO:225]

45 Oligo #B57 Ent338.UP Length: 63
 CATGGCATAT CCAGAACTG ATTACAAGGA CGACGATGAC AAGGCTAACT
 GCTCTATAAT GAT [SEQ ID NO:226]

50 09L2Q6S1.REQ Length: 000032
 AATTCGGGCT TAAACTGCAA TTCTATCTGT CT [SEQ ID NO:227]

55 09L2Q6S3.REQ Length: 000037
 CTCAAGGGTA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ ID NO:228]
 117S2.REQ Length: 000032

60 TCTCTTGAGC AAGCGCAGGA ACAACAGTAA TA [SEQ ID NO:229]

95

19I0L3A1.REQ Length: 000040
CATGGCAAAC TGCTCTATAA TACTCGATGA AGCAATACAT [SEQ ID NO:230]

5 19I0L3A4.REQ Length: 000045
CTTTAAGTGA TGTATTGCTT CATCGAGTAT TATAGAGCAG TTTGC [SEQ. ID
NO.:231]

10 20P23A1.REQ Length: 000040
CATGGCAAAC TGCTCTATAA TGCCAGATGA AGCAATACAT [SEQ. ID
NO.:232]

15 20P23A4.REQ Length: 000045
CTTTAAGTGA TGTATTGCTT CATCTGGCAT TATAGAGCAG TTTGC [SEQ. ID
NO.:233]

20 23L1.REQ Length: 000040
CATGGCaAAC TGCTCTATAA TGATCGATGA AactgATACAT [SEQ. ID
NO.:234]

25 23L4.REQ Length: 000045
CTTTAAGTGA TGTATcagTT CATCGATCAT TATAGAGCAG TTtGC [SEQ. ID
NO.:235]

30 29I4S7S2.REQ Length: 000036
CACTTAAAGA TACCACCTAA CCCTAGCCTG GACAGT [SEQ. ID NO.:236]

35 29I4S7S5.REQ Length: 000036
GAGGTTAGCA CTGTCCAGGC TAGGGTTAGG TGGTAT [SEQ. ID NO.:237]

40 38A5V6S3.REQ Length: 000027
GCTAACCTCA ATTCCGAAGA CGTCTCT [SEQ. ID NO.:238]

45 38A5V6S6.REQ Length: 000018
AGAGACGTCT TCGGAATT [SEQ. ID NO.:239]

50 50D51S1.REQ Length: 000036
ATCCTGATGG ACTCCAACCT TCGAACTCCA AACCTG [SEQ. ID NO.:240]

50 50D51S4.REQ Length: 000027
AGTTCGAAGG TTGGAGTCCA TCAGGAT [SEQ. ID NO.:241]

55 5VYWPTT3.REQ Length: 000048
GTTCCCTATT GGACGGCCCC TCCCTCTCGA ACACCAATCA CGATCAAG [SEQ. ID
NO.:242]

60 5VYWPTT7.REQ Length: 000048

CGTGATTGGT GTTCGAGAGG GAGGGGCCGT CCAATAGGGA ACACATGG [SEQ. ID
NO.:243]

5 62P3H5S2.REQ Length: 000024
CTCGCATTC CACATGCTTC TAAG [SEQ. ID NO.:244]

62P63H2.REQ Length: 000024
10 CTCGCATTCC CACATGCTGT CAAG [SEQ. ID NO.:245]

62P63H5.REQ Length: 000024
15 ATGTGGGAAT GCGAGCAGGT TTGG [SEQ. ID NO.:246]

65S67Q6.REQ Length: 000020
TTTTCTAATT GCTTAGAAGC [SEQ. ID NO.:247]

20 67Q3.REQ Length: 000015
CAATTAGAAA ATGCA [SEQ. ID NO.:248]

67Q6.REQ Length: 000021
25 TTTTCTAATT GCTTGACAGC [SEQ. ID NO.:249]

76P1.REQ Length: 000021
30 TCAGGTATTG AGCCAATTCT T [SEQ. ID NO.:250]

76P5.REQ Length: 000019
TGGCTCAATA CCTGATGCA [SEQ. ID NO.:251]

35 79S2.REQ Length: 000018
TCTAATCTCC AACCATGT [SEQ. ID NO.:252]

40 79S6.REQ Length: 000018
TTGGAGATTA GAAAGAAT [SEQ. ID NO.:253]

9L2Q67S3.REQ Length: 000037
45 CTCAAGAGAA GACAGATAGA ATTGCAGTTT AAGCCGG [SEQ. ID NO.:254]

9LQS1181.REQ Length: 000043
50 AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID
NO.:256]

9LQS1183.REQ Length: 000043
55 AGCTTATTAA AGGGTAGACA GATAGAATTG CAGTTTAAGC CGG [SEQ. ID
NO.:257]

S9L2Q6S1.REQ Length: 000043
60 AATTCCGGCT TAAACTGCAA TTCTATCTGT CTACCCTTTA ATA [SEQ. ID

NO.:258]

5

TABLE 3POLYPEPTIDES

10 **PEPTIDE #1;** pMON5988 (Example 43); (15-125)hIL-3

| | | | | | | | | | | | | | |
|----|---|-----|-----|-----|-----|-----|-----|-----|----------------|-----|-----|-----|-----|
| 15 | Asn | Cys | Ser | Asn | Met | Ile | Asp | Glu | Ile | Ile | Thr | His | Leu |
| | 15 | | | | | 20 | | | | | 25 | | |
| 15 | Lys | Gln | Pro | Pro | Leu | Pro | Leu | Leu | Asp | Phe | Asn | Asn | Gly |
| | 30 | | | | | | | 35 | | | | 40 | |
| | Glu | Asp | Gln | Asp | Ile | Leu | Met | Glu | Asn | Asn | Leu | Arg | Asn |
| | 45 | | | | | | | 50 | | | | 55 | |
| 20 | Leu | Glu | Ala | Phe | Asn | Arg | Ala | Val | Lys | Ser | Leu | Gln | Ser |
| | 60 | | | | | | | 65 | | | | 70 | |
| | Ala | Ile | Glu | Ser | Ile | Leu | Lys | Asn | Leu | Leu | Pro | Cys | Leu |
| | 75 | | | | | | | 80 | | | | 85 | |
| 25 | Ala | Thr | Ala | Ala | Pro | Thr | Arg | His | Pro | Ile | His | Ile | Gly |
| | 90 | | | | | | | 95 | | | | 100 | |
| 30 | Asp | Trp | Asn | Glu | Phe | Arg | Arg | Lys | Leu | Thr | Phe | Tyr | Thr |
| | 105 | | | | | | | 110 | | | | 115 | |
| | Leu | Glu | Asn | Ala | Gln | Ala | Gln | Gln | [SEQ ID NO:65] | | | | |
| | 120 | | | | | | 125 | | | | | | |
| 35 | Asn | Cys | Ser | Asn | Met | Ile | Asp | Glu | Ile | Ile | Thr | His | Leu |
| | | | | | | | | | | | | | |
| | Lys | Gln | Pro | Pro | Leu | Pro | Leu | Leu | Asp | Phe | Asn | Asn | Gly |
| 40 | | | | | | | | | | | | | |
| | Glu | Asp | Gln | Asp | Ile | Leu | Met | Glu | Asn | Asn | Leu | Arg | Asn |
| | | | | | | | | | | | | | |
| 45 | Leu | Glu | Ala | Phe | Asn | Arg | Ala | Val | Lys | Ser | Leu | Gln | Ser |
| | | | | | | | | | | | | | |
| | Ala | Ile | Glu | Ser | Ile | Leu | Lys | Asn | Leu | Leu | Pro | Cys | Leu |
| | | | | | | | | | | | | | |
| 50 | Ala | Thr | Ala | Ala | Pro | Thr | Arg | His | Pro | Ile | His | Ile | Gly |
| | | | | | | | | | | | | | |
| | Asp | Trp | Asn | Glu | Phe | Arg | Arg | Lys | Leu | Thr | Phe | Tyr | Thr |
| | | | | | | | | | | | | | |
| 55 | Leu | Glu | Asn | Ala | Gln | Ala | Gln | Gln | [SEQ ID NO:65] | | | | |
| | | | | | | | | | | | | | |
| 60 | PEPTIDE #2; pMON13344 (Example 8); (15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A and 45V); | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|----|--|-----|-----|-----|-----|-----|-----|-----|----------------|-----|-----|-----|-----|-----|
| | | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ile | Ile | His | His | Leu |
| | | 15 | | | | | 20 | | | | | 25 | | |
| 5 | Lys | Arg | Pro | Pro | Ala | Pro | Leu | Leu | Asp | Pro | Asn | Asn | Leu | Asn |
| | | | 30 | | | | | 35 | | | | | 40 | Ala |
| | Glu | Asp | Val | Asp | Ile | Leu | Met | Glu | Asn | Asn | Leu | Arg | Arg | Pro |
| | | | 45 | | | | | 50 | | | | | 55 | Asn |
| 10 | Leu | Glu | Ala | Phe | Asn | Arg | Ala | Val | Lys | Ser | Leu | Gln | Asn | Ala |
| | | | 60 | | | | | 65 | | | | | 70 | Ser |
| | Ala | Ile | Glu | Ser | Ile | Leu | Lys | Asn | Leu | Leu | Pro | Cys | Leu | Pro |
| 15 | | | 75 | | | | | 80 | | | | | 85 | Leu |
| | Ala | Thr | Ala | Ala | Pro | Thr | Arg | His | Pro | Ile | His | Ile | Lys | Asp |
| | | | 90 | | | | | 95 | | | | | 100 | Gly |
| 20 | Asp | Trp | Asn | Glu | Phe | Arg | Arg | Lys | Leu | Thr | Phe | Tyr | Leu | Lys |
| | | | 105 | | | | | 110 | | | | | 115 | Thr |
| | Leu | Glu | Asn | Ala | Gln | Ala | Gln | Gln | [SEQ ID NO:66] | | | | | |
| | | | 120 | | | | | 125 | | | | | | |
| 25 | | | | | | | | | | | | | | |
| | PEPTIDE #3; pMON13345 (Example 9); (15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S and 45M); | | | | | | | | | | | | | |
| 30 | | | | | | | | | | | | | | |
| | | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ile | Ile | His | His | Leu |
| | | 15 | | | | | 20 | | | | | 25 | | |
| | Lys | Arg | Pro | Pro | Asn | Pro | Leu | Leu | Asp | Pro | Asn | Asn | Leu | Asn |
| 35 | | | 30 | | | | | 35 | | | | | 40 | Ser |
| | Glu | Asp | Met | Asp | Ile | Leu | Met | Glu | Asn | Asn | Leu | Arg | Arg | Pro |
| | | | 45 | | | | | 50 | | | | | 55 | Asn |
| 40 | Leu | Glu | Ala | Phe | Asn | Arg | Ala | Val | Lys | Ser | Leu | Gln | Asn | Ala |
| | | | 60 | | | | | 65 | | | | | 70 | Ser |
| | Ala | Ile | Glu | Ser | Ile | Leu | Lys | Asn | Leu | Leu | Pro | Cys | Leu | Pro |
| | | | 75 | | | | | 80 | | | | | 85 | Leu |
| 45 | Ala | Thr | Ala | Ala | Pro | Thr | Arg | His | Pro | Ile | His | Ile | Lys | Asp |
| | | | 90 | | | | | 95 | | | | | 100 | Gly |
| | Asp | Trp | Asn | Glu | Phe | Arg | Arg | Lys | Leu | Thr | Phe | Tyr | Leu | Lys |
| 50 | | | 105 | | | | | 110 | | | | | 115 | Thr |
| | Leu | Glu | Asn | Ala | Gln | Ala | Gln | Gln | [SEQ ID NO:67] | | | | | |
| | | | 120 | | | | | 125 | | | | | | |
| 55 | | | | | | | | | | | | | | |
| | PEPTIDE #4; pMON13346 (Example 10); (15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S and 45M); | | | | | | | | | | | | | |
| 60 | | | | | | | | | | | | | | |
| | | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ile | Ile | His | His | Leu |
| | | 15 | | | | | 20 | | | | | 25 | | |

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 30 35 40

5
 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55

10
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

15
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100

20
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]
 120 125

25 **PEPTIDE #5;** pMON13347 (Example 12); (15-125)hIL-3 (51R, 55L, 59L,
 62V,
 67N and 69E);

30
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 35 40

35
 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

40
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

45
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115

50
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]
 120 125

55 **PEPTIDE #6;** pMON13348 (Example 13); (15-125)hIL-3 (51R, 55L, 60S,
 62V,
 67N and 69E);

60
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

100

| | 30 | | 35 | | 40 | |
|--|-----------------------------|---------------------|---------------------|-------------------------|-----------------|------------|
| 5 | Glu Asp Gln Asp Ile Leu Met | 45 | Glu Arg Asn Leu Arg | 50 | Leu Pro Asn 55 | |
| | Leu Glu Ser Phe Val Arg Ala | 60 | Val Lys Asn Leu Glu | 65 | Asn Ala Ser 70 | |
| 10 | Ala Ile Glu Ser Ile Leu Lys | 75 | Asn Leu Leu Pro Cys | 80 | Leu Pro Leu 85 | |
| | Ala Thr Ala Ala Pro Thr Arg | 90 | His Pro Ile His Ile | 95 | Lys Asp Gly 100 | |
| 15 | Asp Trp Asn Glu Phe Arg Arg | 105 | Lys Leu Thr Phe Tyr | 110 | Leu Lys Thr 115 | |
| 20 | Leu Glu Asn Ala Gln Ala Gln | 120 | Gln [SEQ ID NO:70] | 125 | | |
| PEPTIDE #7; pMON13349 (Example 14); (15-125)hIL-3 (51R, 55T, 59L, 62V, 67H and 69E); | | | | | | |
| 25 | | Asn Cys Ser Asn Met | 15 | Ile Asp Glu Ile Ile Thr | 20 | His Leu 25 |
| 30 | Lys Gln Pro Pro Leu Pro Leu | 30 | Leu Asp Phe Asn Asn | 35 | Leu Asn Gly 40 | |
| | Glu Asp Gln Asp Ile Leu Met | 45 | Glu Arg Asn Leu Arg | 50 | Thr Pro Asn 55 | |
| 35 | Leu Leu Ala Phe Val Arg Ala | 60 | Val Lys His Leu Glu | 65 | Asn Ala Ser 70 | |
| | Ala Ile Glu Ser Ile Leu Lys | 75 | Asn Leu Leu Pro Cys | 80 | Leu Pro Leu 85 | |
| 40 | Ala Thr Ala Ala Pro Thr Arg | 90 | His Pro Ile His Ile | 95 | Lys Asp Gly 100 | |
| | Asp Trp Asn Glu Phe Arg Arg | 105 | Lys Leu Thr Phe Tyr | 110 | Leu Lys Thr 115 | |
| 45 | Leu Glu Asn Ala Gln Ala Gln | 120 | Gln [SEQ ID NO:71] | 125 | | |
| 50 | | | | | | |
| PEPTIDE #8; pMON13350 (Example 16); (15-125)hIL-3 (73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A and 105Q); | | | | | | |
| 55 | | Asn Cys Ser Asn Met | 15 | Ile Asp Glu Ile Ile Thr | 20 | His Leu 25 |
| 60 | Lys Gln Pro Pro Leu Pro Leu | | Leu Asp Phe Asn Asn | | Leu Asn Gly | |

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| | 30 | 35 | 40 |
|----|--|---------------------|-------------|
| | Glu Asp Gln Asp Ile Leu Met | Glu Asn Asn Leu Arg | Arg Pro Asn |
| | 45 | 50 | 55 |
| 5 | Leu Glu Ala Phe Asn Arg Ala | Val Lys Ser Leu Gln | Asn Ala Ser |
| | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| 10 | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| 15 | Asp Trp Gln Glu Phe Arg Arg | Lys Leu Thr Phe Tyr | Leu Lys Thr |
| | 105 | 110 | 115 |
| | Leu Glu Asn Ala Gln Ala Gln | Gln [SEQ ID NO:72] | |
| | 120 | 125 | |
| 20 | PEPTIDE #9; pMON13355 (Example 17); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A and 105Q); | | |
| 25 | Asn Cys Ser Asn Met | Ile Asp Glu Ile Ile | Thr His Leu |
| | 15 | 20 | 25 |
| | Lys Gln Pro Pro Leu Pro Leu | Leu Asp Phe Asn Asn | Leu Asn Gly |
| | 30 | 35 | 40 |
| 30 | Glu Asp Gln Asp Ile Leu Met | Glu Asn Asn Leu Arg | Arg Pro Asn |
| | 45 | 50 | 55 |
| | Leu Glu Ala Phe Asn Arg Ala | Val Lys Ser Leu Gln | Asn Ala Ser |
| 35 | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Val Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| 40 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Thr Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| | Asp Trp Gln Glu Phe Arg Arg | Lys Leu Thr Phe Tyr | Leu Lys Thr |
| | 105 | 110 | 115 |
| 45 | Leu Glu Asn Ala Gln Ala Gln | Gln [SEQ ID NO:73] | |
| | 120 | 125 | |
| 50 | PEPTIDE #10; pMON13352 (Example 19); (15-125)hIL-3 (109E, 116V, 120Q and 123E); | | |
| 55 | Asn Cys Ser Asn Met | Ile Asp Glu Ile Ile | Thr His Leu |
| | 15 | 20 | 25 |
| | Lys Gln Pro Pro Leu Pro Leu | Leu Asp Phe Asn Asn | Leu Asn Gly |
| | 30 | 35 | 40 |
| 60 | Glu Asp Gln Asp Ile Leu Met | Glu Asn Asn Leu Arg | Arg Pro Asn |
| | 45 | 50 | 55 |

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Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]
 120 125
PEPTIDE #11; pMON13354 (Example 20); (15-125)hIL-3 (109E, 116V,
 20 117S, 120H
 and 123E);
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 25 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 35 40
 30 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 40 Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 105 110 115
 45 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:75]
 120 125
PEPTIDE #12; pMON13360 (Example 21); (15-125)hIL-3 (73G, 76A,
 79R, 82Q,
 50 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 55 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 35 40
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 60 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

103

| | 60 | 65 | 70 |
|----|---|---------------------|-------------|
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| 5 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| 10 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr |
| | 105 | 110 | 115 |
| | Leu Glu Gln Ala Gln Glu Gln | Gln [SEQ. NO:76] | |
| | 120 | 125 | |
| 15 | | | |
| | PEPTIDE #13: pMON13361 (Example 22); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E); | | |
| 20 | Asn Cys Ser Asn Met | Ile Asp Glu Ile Ile | Thr His Leu |
| | 15 | 20 | 25 |
| | Lys Gln Pro Pro Leu Pro Leu | Leu Asp Phe Asn Asn | Leu Asn Gly |
| 25 | 30 | 35 | 40 |
| | Glu Asp Gln Asp Ile Leu Met | Glu Asn Asn Leu Arg | Arg Pro Asn |
| | 45 | 50 | 55 |
| 30 | Leu Glu Ala Phe Asn Arg Ala | Val Lys Ser Leu Gln | Asn Ala Ser |
| | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Val Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| 35 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Thr Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr |
| 40 | 105 | 110 | 115 |
| | Leu Glu Gln Ala Gln Glu Gln | Gln [SEQ ID NO:77] | |
| | 120 | 125 | |
| 45 | | | |
| | PEPTIDE #14: pMON13362 (Example 23); (15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E); | | |
| 50 | Asn Cys Ser Asn Met | Ile Asp Glu Ile Ile | Thr His Leu |
| | 15 | 20 | 25 |
| | Lys Gln Pro Pro Leu Pro Leu | Leu Asp Phe Asn Asn | Leu Asn Gly |
| 55 | 30 | 35 | 40 |
| | Glu Asp Gln Asp Ile Leu Met | Glu Asn Asn Leu Arg | Arg Pro Asn |
| | 45 | 50 | 55 |
| 60 | Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser | | |

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| | 60 | 65 | 70 |
|----|---|---------------------|-------------|
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Val Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| 5 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Thr Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| 10 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Ser |
| | 105 | 110 | 115 |
| | Leu Glu His Ala Gln Glu Gln | [SEQ ID NO:78] | |
| | 120 | 125 | |
| 15 | PEPTIDE #15; pMON13363 (Example 24); (15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E); | | |
| | Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu |
| 20 | 15 | 20 | 25 |
| | Lys Arg Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ala |
| | 30 | 35 | 40 |
| 25 | Glu Asp Val Asp Ile Leu Met | Glu Arg Asn Leu Arg | Leu Pro Asn |
| | 45 | 50 | 55 |
| | Leu Glu Ser Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |
| 30 | Ala Ile Glu Ser Ile Leu Lys | Asn Leu Leu Pro Cys | Leu Pro Leu |
| | 75 | 80 | 85 |
| 35 | Ala Thr Ala Ala Pro Thr Arg | His Pro Ile His Ile | Lys Asp Gly |
| | 90 | 95 | 100 |
| | Asp Trp Asn Glu Phe Arg Arg | Lys Leu Thr Phe Tyr | Leu Lys Thr |
| | 105 | 110 | 115 |
| 40 | Leu Glu Asn Ala Gln Ala Gln | [SEQ ID NO:79] | |
| | 120 | 125 | |
| 45 | PEPTIDE #16; pMON13364 (Example 25); (15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E); | | |
| | Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu |
| | 15 | 20 | 25 |
| 50 | Lys Arg Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ser |
| | 30 | 35 | 40 |
| 55 | Glu Asp Met Asp Ile Leu Met | Glu Arg Asn Leu Arg | Thr Pro Asn |
| | 45 | 50 | 55 |
| | Leu Leu Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |
| 60 | Ala Ile Glu Ser Ile Leu Lys | Asn Leu Leu Pro Cys | Leu Pro Leu |
| | 75 | 80 | 85 |

105

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100

5 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:80]
 120 125

10

PEPTIDE #17; pMON13365 (Example 26); (15-125)hIL-3 (18I, 25H,
 29V, 32A,
 15 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E);

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

20 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

25 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

30 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100

35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:81]
 120 125

40

PEPTIDE #18; pMON13298 (Example 27); Met-Ala-(15-125)hIL-3 (73G,
 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and
 123E);

45 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 35 40

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55

55 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

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| | 90 | 95 | 100 |
|----|---|-----|-----|
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | |
| | 105 | 110 | 115 |
| 5 | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82] | | |
| | 120 | 125 | |
| 10 | PEPTIDE #19; pMON13299 (Example 28); Met-Ala-(15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E); | | |
| 15 | Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu | | |
| | 15 | 20 | 25 |
| | Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly | | |
| | 30 | 35 | 40 |
| 20 | Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn | | |
| | 45 | 50 | 55 |
| | Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser | | |
| 25 | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser | | |
| | 75 | 80 | 85 |
| 30 | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly | | |
| | 90 | 95 | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | |
| | 105 | 110 | 115 |
| 35 | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83] | | |
| | 120 | 125 | |
| 40 | PEPTIDE #20; pMON13300 (Example 29); Met-Ala-(15-125)hIL-3 (73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E); | | |
| | Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu | | |
| | 15 | 20 | 25 |
| 45 | Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly | | |
| | 30 | 35 | 40 |
| | Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn | | |
| 50 | 45 | 50 | 55 |
| | Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser | | |
| | 60 | 65 | 70 |
| 55 | Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser | | |
| | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly | | |
| | 90 | 95 | 100 |
| 60 | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser | | |

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| | | | | | |
|---|---|--|-----|--|-----|
| | 105 | | 110 | | 115 |
| | Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:84] | | | | |
| 5 | 120 | | 125 | | |
| PEPTIDE #21; pMON13301 (Example 30); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E); | | | | | |
| 10 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala | | | | |
| 15 | 30 | | 35 | | 40 |
| | Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| 20 | Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser | | | | |
| | 60 | | 65 | | 70 |
| | Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu | | | | |
| | 75 | | 80 | | 85 |
| 25 | Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr | | | | |
| 30 | 105 | | 110 | | 115 |
| | Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:85] | | | | |
| | 120 | | 125 | | |
| PEPTIDE #22; pMON13302 (Example 31); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E); | | | | | |
| 35 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| 40 | Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser | | | | |
| | 30 | | 35 | | 40 |
| | Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn | | | | |
| 45 | 45 | | 50 | | 55 |
| | Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser | | | | |
| | 60 | | 65 | | 70 |
| 50 | Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu | | | | |
| | 75 | | 80 | | 85 |
| | Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly | | | | |
| 55 | 90 | | 95 | | 100 |
| | Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr | | | | |
| | 105 | | 110 | | 115 |
| 60 | Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:86] | | | | |
| | 120 | | 125 | | |

PEPTIDE #23; pMON13303 (Example 32); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E);

```

5  Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
    15                      20                      25

Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
    30                      35                      40

10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
    45                      50                      55

15 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
    60                      65                      70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
    75                      80                      85

20 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
    90                      95                      100

Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
    105                     110                     115

25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:87]
    120                     125

```

PEPTIDE #24; pMON13287 (Example 33); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

```

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
    15                      20                      25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
    30                      35                      40

40 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
    45                      50                      55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
    60                      65                      70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
    75                      80                      85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
    90                      95                      100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
    105                     110                     115

55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:88]
    120                     125

```

PEPTIDE #25; pMON13288 (Example 34); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and

109

123E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70

15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]
 120 125

25

PEPTIDE #26; pMON13289 (Example 35); Met-Ala-(15-125)hIL-3 (18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E);

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

35 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 30 35 40

40 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

45 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]
 120 125

55

PEPTIDE #27; pMON13290 (Example 36); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E);

60

110

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 5
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 10
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 15
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 75 80 85
 20
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 25
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:91]
 120 125
PEPTIDE #28; pMON13292 (Example 37); Met-Ala-(15-125)hIL-3 (18I,
 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G,
 30 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and
 123E);
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 35
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 30 35 40
 40
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 45
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 90 95 100
 50
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 55
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]
 120 125
PEPTIDE #29; pMON13294 (Example 38); Met-Ala-(15-125)hIL-3 (18I,
 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G,
 60 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H
 and 123E);

111

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
45 50 55

10 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
75 80 85

15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
105 110 115

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:93]
120 125

25 **PEPTIDE #30**; pMON13295 (Example 39); Met-Ala-(15-125)hIL-3 (18I,
25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G,
76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H
and 123E);

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

35 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
30 35 40

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

40 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
75 80 85

45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
90 95 100

50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
105 110 115

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:94]
120 125

55 **PEPTIDE #31**; pMON13312 (Example 40); Met-Ala-(15-125)hIL-3 (18I,
25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G,
76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and
123E);

60 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

112

| | 15 | 20 | 25 |
|----|--|---------------------------------|-----|
| | Lys Arg Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn Leu Asn Ser | |
| | 30 | 35 | 40 |
| 5 | Glu Asp Met Asp Ile Leu Met | Glu Arg Asn Leu Arg Thr Pro Asn | |
| | 45 | 50 | 55 |
| 10 | Leu Leu Ala Phe Val Arg Ala | Val Lys His Leu Glu Asn Ala Ser | |
| | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Val Pro Cys Leu Pro Ser | |
| | 75 | 80 | 85 |
| 15 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Thr Ile Lys Ala Gly | |
| | 90 | 95 | 100 |
| 20 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr Leu Val Thr | |
| | 105 | 110 | 115 |
| | Leu Glu Gln Ala Gln Glu Gln | Gln [SEQ ID NO:95] | |
| | 120 | 125 | |
| 25 | PEPTIDE #32; pMON13313 (Example 41); Met-Ala-(15-125)hIL-3 (18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E); | | |
| 30 | Met Ala Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile His His Leu | |
| | 15 | 20 | 25 |
| 35 | Lys Arg Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn Leu Asn Ala | |
| | 30 | 35 | 40 |
| | Glu Asp Val Asp Ile Leu Met | Glu Arg Asn Leu Arg Leu Pro Asn | |
| | 45 | 50 | 55 |
| 40 | Leu Glu Ser Phe Val Arg Ala | Val Lys Asn Leu Glu Asn Ala Ser | |
| | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Val Pro Cys Leu Pro Ser | |
| | 75 | 80 | 85 |
| 45 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Thr Ile Lys Ala Gly | |
| | 90 | 95 | 100 |
| 50 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr Leu Val Ser | |
| | 105 | 110 | 115 |
| | Leu Glu His Ala Gln Glu Gln | Gln [SEQ ID NO:96] | |
| | 120 | 125 | |
| 55 | PEPTIDE #A3; pMON13285 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 50D); | | |
| 60 | Met Ala Asn Cys Ser Asn Met | Ile Asp Glu Ile Ile Thr His Leu | |
| | 15 | 20 | 25 |

113

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40
 5 Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 10 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115
 20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:259]
 120 125

PEPTIDE #A4; pMON13286 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S);

25 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40
 30 Glu Asp **Met Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 35 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 40 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 105 110 115
 45 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:260]
 120 125

50 **PEPTIDE #A5;** pMON13325 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S, 116W);

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 55 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40
 60 Glu Asp **Met Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55

114

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 10 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:261]
 120 125
 15
PEPTIDE #A6; pMON13326 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S,
 50D, 116W);
 20 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40
 25 Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 30 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85
 35 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 90 95 100
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115
 40 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:262]
 120 125
 45 **PEPTIDE #A7;** pMON13330 Met-Ala-IL-3; (42D, 45M, 46S, 50D, 95R,
 98I, 100R, 116W);
 50 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40
 55 Glu Asp **Met Ser** Ile Leu Met **Asp** Asn Asn Leu Arg Arg Pro Asn
 45 50 55
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70
 60 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

115

Ala Thr Ala Ala Pro Thr Arg **Arg** Pro Ile **Ile** Ile **Arg** Asp Gly
 90 95 100

5 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:263]
 120 125

10

PEPTIDE #A8; pMON13329 Met-Ala-(15-125)hIL-3; (42D, 45M, 46S,
 98I, 100R, 116W);

15 Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 15 20 25

20 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn **Asp**
 30 35 40

Glu Asp **Met** **Ser** Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 45 50 55

25 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 60 65 70

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 75 80 85

30 Ala Thr Ala Ala Pro Thr Arg His Pro Ile **Ile** Ile **Arg** Asp Gly
 90 95 100

35 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu **Trp** Thr
 105 110 115

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:406]
 120 125

40

PEPTIDE #B1 Met-Ala-(15-125)hIL-3 pMON13406

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
 15 20 25

45 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

50 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

116

| | | | | | |
|--|---|--|-----|--|-----|
| | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 264] | | | | |
| 5 | 120 | | 125 | | |
| PEPTIDE # B2 Met-Ala-(15-125)hIL-3 pMON13414 | | | | | |
| 10 | Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser | | | | |
| | 30 | | 35 | | 40 |
| 15 | Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| | Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser | | | | |
| 20 | 60 | | 65 | | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| | 75 | | 80 | | 85 |
| 25 | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| | 105 | | 110 | | 115 |
| 30 | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 265] | | | | |
| | 120 | | 125 | | |
| PEPTIDE #B3 Met-Ala-(15-125)hIL-3 pMON13407 | | | | | |
| 35 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser | | | | |
| 40 | 30 | | 35 | | 40 |
| | Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| 45 | Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser | | | | |
| | 60 | | 65 | | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| | 75 | | 80 | | 85 |
| 50 | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| 55 | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 266] | | | | |
| | 120 | | 125 | | |
| 60 | PEPTIDE #B4 Met-Ala-(15-125)hIL-3 pMON13405 | | | | |

117

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
 15 20 25
 5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 10 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 267]
 120 125
 25
 PEPTIDE #B5 Met-Ala-(15-125)hIL-3 pMON13415
 30 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 35 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 45 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 268]
 120 125
 55
 PEPTIDE #B6 Met-Ala-(15-125)hIL-3 pMON13408
 60 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

118

Glu Asp Met Asp Ile Leu Ile Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 5 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 269]
 120 125
 20 PEPTIDE #B7 Met-Ala-(15-125)hIL-3 pMON13409
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 25 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 Glu Asp Met Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 30 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 35 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 270]
 120 125
 45
 PEPTIDE #B8 Met-Ala-(15-125)hIL-3 pMON13410
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 50 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 55 Glu Asp Met Asp Ile Leu Asp Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

119

| | | | | | |
|----|---------|---------------------|-----------------------|-------------|----|
| | 75 | | 80 | | 85 |
| | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| 5 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |
| 10 | Leu Glu | Gln Ala Gln Glu Gln | Gln [SEQ ID NO.: 271] | | |
| | 120 | | 125 | | |

PEPTIDE #B9 Met-Ala-(15-125)hIL-3 pMON13422

| | | | | | |
|----|---------|---------------------|-----------------------|-------------|--|
| 15 | Met Ala | Asn Cys Ser Ile Ala | Ile Asp Glu Ile Ile | His His Leu | |
| | 15 | | 20 | 25 | |
| | Lys Arg | Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ser | |
| | 30 | | 35 | 40 | |
| 20 | Glu Asp | Val Asp Ile Leu Ile | Glu Arg Asn Leu Arg | Thr Pro Asn | |
| | 45 | | 50 | 55 | |
| 25 | Leu Leu | Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser | |
| | 60 | | 65 | 70 | |
| | Gly Ile | Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | |
| | 75 | | 80 | 85 | |
| 30 | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |
| 35 | Leu Glu | Gln Ala Gln Glu Gln | Gln [SEQ ID NO.: 272] | | |
| | 120 | | 125 | | |

40 PEPTIDE #B10 Met-Ala-(15-125)hIL-3 pMON13423

| | | | | | |
|----|---------|---------------------|---------------------|-------------|--|
| | Met Ala | Asn Cys Ser Ile Ile | Ile Asp Glu Ile Ile | His His Leu | |
| | 15 | | 20 | 25 | |
| 45 | Lys Arg | Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ser | |
| | 30 | | 35 | 40 | |
| | Glu Asp | Val Asp Ile Leu Ile | Glu Arg Asn Leu Arg | Thr Pro Asn | |
| | 45 | | 50 | 55 | |
| 50 | Leu Leu | Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser | |
| | 60 | | 65 | 70 | |
| | Gly Ile | Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | |
| | 75 | | 80 | 85 | |
| 55 | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| 60 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |

120

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 273]
 120 125

5

PEPTIDE #B11 Met-Ala-(15-125)hIL-3 pMON13424

10 Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
 15 20 25

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

15 Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 274]
 120 125

PEPTIDE #B12 Met-Ala-(15-125)hIL-3 pMON13425

35 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu
 15 20 25

40 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40

Glu Asp Val Asp Ile Leu Leu Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 275]
 120 125

60

PEPTIDE #B13 Met-Ala-(15-125)hIL-3 pMON13426

121

Met Ala Asn Cys Ser Ile Ala Ile Asp Glu Ile Ile His His Leu
 15 20 25
 5 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 10 Glu Asp Val Asp Ile Leu Asp Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 276]
 120 125
 30 PEPTIDE #B14 Met-Ala-(15-125)hIL-3 pMON13429
 Met Ala Asn Cys Ser Ile Ile Ile Asp Glu Ile Ile His His Leu
 15 20 25
 35 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 Glu Asp Val Asp Ile Leu Asp Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 40 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 50 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 277]
 120 125
 55 PEPTIDE #B15 Met-Ala-(15-125)hIL-3 pMON13368
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 60 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

122

| | 30 | 35 | 40 |
|----|-----------------------------|----------------------|-------------|
| | Glu Asp Met Asp Ile Leu Met | Glu Arg Asn Leu Arg | Leu Pro Asn |
| 5 | 45 | 50 | 55 |
| | Leu Leu Ala Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |
| 10 | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| 15 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr |
| | 105 | 110 | 115 |
| | Leu Glu Gln Ala Gln Glu Gln | Gln [SEQ ID NO: 278] | |
| 20 | 120 | 125 | |

PEPTIDE #B16 Met-Ala-(15-125)hIL-3 pMON13380

| | | | |
|----|-----------------------------|----------------------|-------------|
| 25 | Met Ala Asn Cys Ser Ile Met | Ile Asp Glu Ala Ile | His His Leu |
| | 15 | 20 | 25 |
| | Lys Val Pro Pro Ala Pro Leu | Leu Asp Ser Asn Asn | Leu Asn Ser |
| | 30 | 35 | 40 |
| 30 | Glu Asp Met Asp Ile Leu Met | Glu Arg Asn Leu Arg | Leu Pro Asn |
| | 45 | 50 | 55 |
| | Leu Leu Ala Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser |
| 35 | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| 40 | 90 | 95 | 100 |
| | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Gln Phe Tyr | Leu Val Thr |
| | 105 | 110 | 115 |
| 45 | Leu Glu Gln Ala Gln Glu Gln | Gln [SEQ ID NO. 279] | |
| | 120 | 125 | |

PEPTIDE #B17 Met-Ala-(15-125)hIL-3 pMON13475

| | | | |
|----|-----------------------------|---------------------|-------------|
| 50 | Met Ala Asn Cys Ser Ile Met | Ile Asp Glu Ala Ile | His His Leu |
| | 15 | 20 | 25 |
| 55 | Lys Arg Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Asp |
| | 30 | 35 | 40 |
| | Glu Asp Val Ser Ile Leu Met | Asp Arg Asn Leu Arg | Leu Pro Asn |
| | 45 | 50 | 55 |
| 60 | Leu Glu Ser Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |

123

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 280]
 120 125
 15 PEPTIDE #B18 Met-Ala-(15-125)hIL-3 pMON13366
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asn
 30 35 40
 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 281]
 120 125
 40
 PEPTIDE #B19 Met-Ala-(15-125)hIL-3 pMON13367
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 45 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 50 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 60 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

124

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 5 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 282]
 120 125
 PEPTIDE #B20 Met-Ala-(15-125)hIL-3 pMON13369
 10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 15 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 20 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 283]
 120 125
 35 PEPTIDE #B21 Met-Ala-(15-125)hIL-3 pMON13370
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 50 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 284]
 120 125
 60

125

PEPTIDE #B22 Met-Ala-(15-125)hIL-3 pMON13378

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 10 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 285]
 120 125

PEPTIDE #B23 Met-Ala-(15-125)hIL-3 pMON13374

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 35 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 40 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 286]
 120 125

55 PEPTIDE #B24 Met-Ala-(15-119)hIL-3 pMON13375

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 60 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

126

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu [SEQ ID NO.: 287]
 119

20 PEPTIDE #B25 Met-Asp-(15-119)hIL-3 pMON13376
 Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 25 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 30 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu [SEQ ID NO.: 288]
 119
 45

PEPTIDE #B26 Met-Ala-(15-125)hIL-3 pMON13377
 50 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 55 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 60 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

127

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 289]
120 125

15 PEPTIDE #B27 Met-Asp-(15-119)hIL-3 pMON13378

Met Asp Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu [SEQ ID NO.: 290]
119

40

PEPTIDE #B28 Met-Ala-(15-125)hIL-3 pMON13379

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

45 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
30 35 40

50 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Gln Phe Tyr Leu Val Thr

128

105

110

115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 291]
 120 125

5

PEPTIDE #B29 Met-Ala-(15-125)hIL-3 pMON13385

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

15 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 292]
 120 125

PEPTIDE #B30 Met-Ala-(15-125)hIL-3 pMON13381

35

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

40 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

45 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Trp Pro Cys Leu Pro Ser
 75 80 85

50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100

55 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 293]
 120 125

60

PEPTIDE #B31 Met-Ala-(15-125)hIL-3 pMON13383

129

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-------------------|-----|-----|-----|-----|-----|-----|
| | Met | Ala | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ala | Ile | His | His | Leu |
| | | | 15 | | | | | 20 | | | | | 25 | | |
| 5 | Lys | Arg | Pro | Pro | Ala | Pro | Leu | Leu | Asp | Pro | Asn | Asn | Leu | Asn | Asp |
| | | | 30 | | | | | 35 | | | | | 40 | | |
| | Glu | Asp | Val | Ser | Ile | Leu | Met | Asp | Arg | Asn | Leu | Arg | Leu | Pro | Asn |
| | | | 45 | | | | | 50 | | | | | 55 | | |
| 10 | Leu | Glu | Ser | Phe | Val | Arg | Ala | Val | Lys | Asn | Leu | Glu | Asn | Ala | Ser |
| | | | 60 | | | | | 65 | | | | | 70 | | |
| | Gly | Ile | Glu | Ala | Ile | Leu | Arg | Asn | Leu | Gln | Pro | Cys | Leu | Pro | Ser |
| 15 | | | 75 | | | | | 80 | | | | | 85 | | |
| | Ala | Thr | Ala | Ala | Pro | Ser | Arg | His | Pro | Ile | Ile | Ile | Lys | Ala | Gly |
| | | | 90 | | | | | 95 | | | | | 100 | | |
| 20 | Asp | Trp | Gln | Glu | Phe | Arg | Glu | Lys | Leu | Gln | Phe | Tyr | Leu | Val | Thr |
| | | | 105 | | | | | 110 | | | | | 115 | | |
| | Leu | Glu | Gln | Ala | Gln | Glu | Gln | Gln | [SEQ ID NO.: 294] | | | | | | |
| 25 | | | 120 | | | | | 125 | | | | | | | |

PEPTIDE #B32 Met-Ala-(15-125)hIL-3 pMON13384

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-------------------|-----|-----|-----|-----|-----|-----|
| 30 | Met | Ala | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ile | Ile | His | His | Leu |
| | | | 15 | | | | | 20 | | | | | 25 | | |
| | Lys | Arg | Pro | Pro | Ala | Pro | Leu | Leu | Asp | Pro | Asn | Asn | Leu | Asn | Ala |
| | | | 30 | | | | | 35 | | | | | 40 | | |
| 35 | Glu | Asp | Val | Asp | Ile | Leu | Met | Glu | Arg | Asn | Leu | Arg | Leu | Pro | Asn |
| | | | 45 | | | | | 50 | | | | | 55 | | |
| | Leu | Glu | Ser | Phe | Val | Arg | Ala | Val | Lys | Asn | Leu | Glu | Asn | Ala | Ser |
| 40 | | | 60 | | | | | 65 | | | | | 70 | | |
| | Gly | Ile | Glu | Ala | Ile | Leu | Arg | Asn | Leu | Gln | Pro | Cys | Leu | Pro | Ser |
| | | | 75 | | | | | 80 | | | | | 85 | | |
| 45 | Ala | Thr | Ala | Ala | Pro | Ser | Arg | His | Pro | Ile | Ile | Ile | Lys | Ala | Gly |
| | | | 90 | | | | | 95 | | | | | 100 | | |
| | Asp | Trp | Gln | Glu | Phe | Arg | Glu | Lys | Leu | Gln | Phe | Tyr | Leu | Val | Thr |
| | | | 105 | | | | | 110 | | | | | 115 | | |
| 50 | Leu | Glu | Gln | Ala | Gln | Glu | Gln | Gln | [SEQ ID NO.: 295] | | | | | | |
| | | | 120 | | | | | 125 | | | | | | | |

55 PEPTIDE #B33 Met-Ala-(15-125)hIL-3 pMON13388

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Met | Ala | Asn | Cys | Ser | Ile | Met | Ile | Asp | Glu | Ile | Ile | His | His | Leu |
| | | | 15 | | | | | 20 | | | | | 25 | | |
| 60 | Lys | Arg | Pro | Pro | Ala | Pro | Leu | Leu | Asp | Pro | Asn | Asn | Leu | Asn | Ala |
| | | | 30 | | | | | 35 | | | | | 40 | | |

130

Glu Asp Val Asp Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn
 45 50 55
 5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 15 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 296]
 120 125
 20
 PEPTIDE #B34 Met-Ala-(15-125)hIL-3 pMON13389
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 25 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 30 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 297]
 120 125
 45
 PEPTIDE #B35 Met-Ala-(15-125)hIL-3 pMON13391
 50 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 55 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 60 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

131

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 85

5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 298]
120 125

15 PEPTIDE #B36 Met-Ala-(15-125)hIL-3 pMON13392

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
15 20 25

20 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

25 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 299]
120 125

40 PEPTIDE #B37 Met-Ala-(15-125)hIL-3 pMON13393

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
15 20 25

45 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
30 35 40

50 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
60 65 70

55 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
90 95 100

60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

132

| | | | | | |
|----|---|--|-----|--|-----|
| | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 300] | | | | |
| 5 | 120 | | 125 | | |
| | PEPTIDE #B38 Met-Ala-(15-125)hIL-3 pMON13394 | | | | |
| 10 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp | | | | |
| | 30 | | 35 | | 40 |
| 15 | Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| | Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser | | | | |
| 20 | 60 | | 65 | | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| | 75 | | 80 | | 85 |
| 25 | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| | 105 | | 110 | | 115 |
| 30 | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 301] | | | | |
| | 120 | | 125 | | |
| | PEPTIDE #B39 Met-Ala-(15-125)hIL-3 pMON13395 | | | | |
| 35 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp | | | | |
| 40 | 30 | | 35 | | 40 |
| | Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| 45 | Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser | | | | |
| | 60 | | 65 | | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| 50 | 75 | | 80 | | 85 |
| | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| 55 | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 302] | | | | |
| | 120 | | 125 | | |
| 60 | PEPTIDE #B40 Met-Ala-(15-125)hIL-3 pMON13396 | | | | |

133

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

5 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

10 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85

15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Arg Met Gly
 90 95 100

20 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 303]
 120 125

25

PEPTIDE #B41 Met-Ala-(15-125)hIL-3 pMON13397

30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40

35 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70

40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Trp Pro Cys Leu Pro Ser
 75 80 85

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Arg Met Gly
 90 95 100

45 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115

50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 304]
 120 125

PEPTIDE #B42 Met-Ala-(15-125)hIL-3 pMON13398

55 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25

60 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40

134

Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 10 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 15 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 305]
 120 125
 20 PEPTIDE #B43 Met-Ala-(15-125)hIL-3 pMON13399
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 25 Lys Val Pro Pro Arg Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 30 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 40 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 306]
 120 125
 45
 PEPTIDE #B44 Met-Ala-(15-119)hIL-3 pMON13404
 50 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 55 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

135

| | | | | | |
|----|---------|---------------------|---------------------|-------------|----|
| | 75 | | 80 | | 85 |
| | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| 5 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Gln Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |
| 10 | Leu Glu | [SEQ ID NO.: 307] | | | |
| | 119 | | | | |

PEPTIDE #B45 Met-Ala-(15-125)hIL-3 pMON13387

| | | | | | |
|----|---------|---------------------|-----------------------|-------------|--|
| 15 | Met Ala | Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu | |
| | 15 | | 20 | 25 | |
| | Lys Arg | Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ala | |
| | 30 | | 35 | 40 | |
| 20 | Glu Asp | Val Asp Ile Leu Met | Asp Arg Asn Leu Arg | Leu Pro Asn | |
| | 45 | | 50 | 55 | |
| 25 | Leu Glu | Ser Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser | |
| | 60 | | 65 | 70 | |
| | Gly Ile | Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | |
| | 75 | | 80 | 85 | |
| 30 | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |
| 35 | Leu Glu | Gln Ala Gln Glu Gln | Gln [SEQ ID NO.: 308] | | |
| | 120 | | 125 | | |

40 PEPTIDE #B46 Met-Ala-(15-125)hIL-3 pMON13416

| | | | | | |
|----|---------|---------------------|---------------------|-------------|--|
| | Met Ala | Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu | |
| | 15 | | 20 | 25 | |
| 45 | Lys Arg | Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Asp | |
| | 30 | | 35 | 40 | |
| | Glu Asp | Val Ser Ile Leu Met | Glu Arg Asn Leu Arg | Leu Pro Asn | |
| | 45 | | 50 | 55 | |
| 50 | Leu Glu | Ser Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser | |
| | 60 | | 65 | 70 | |
| | Gly Ile | Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | |
| | 75 | | 80 | 85 | |
| 55 | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | |
| | 90 | | 95 | 100 | |
| 60 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | |
| | 105 | | 110 | 115 | |

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Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 309]
 120 125

5

PEPTIDE #B47 Met-Ala-(15-125)hIL-3 pMON13417

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 10 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 15 Glu Asp Met Ser Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 310]
 120 125

PEPTIDE #B48 Met-Ala-(15-125)hIL-3 pMON13420

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 35 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 40 Glu Asp Val Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 50 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 55 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 311]
 120 125

60 PEPTIDE #B49 Met-Ala-(15-125)hIL-3 pMON13421

137

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 5 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Ser Asn
 45 50 55
 10 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.:331]
 120 125
 25
 PEPTIDE #B50 Met-Ala-(15-125)hIL-3 pMON13432
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ala Ile His His Leu
 15 20 25
 30 Lys Arg Pro Pro Ala Pro Ser Leu Asp Pro Asn Asn Leu Asn Asp
 30 35 40
 Glu Asp Met Ser Ile Leu Met Asp Arg Asn Leu Arg Leu Pro Asn
 45 50 55
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 60 65 70
 40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 45 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 50 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 312]
 120 125
 PEPTIDE #B51 Met-Ala-(15-125)hIL-3 pMON13382
 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 30 35 40
 60 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

138

| | | | | | |
|--|-------------------------------------|---------------------------------|-------------|----|----|
| | 45 | | 50 | | 55 |
| | Leu Glu Ser Phe Val Arg Ala | Val Lys Asn Leu Glu Asn Ala Ser | | | |
| | 60 | 65 | 70 | | |
| 5 | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | | |
| | 75 | 80 | 85 | | |
| 10 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | | |
| | 90 | 95 | 100 | | |
| | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Gln Phe Tyr | Leu Trp Thr | | |
| | 105 | 110 | 115 | | |
| 15 | Leu Glu Gln Ala Gln Glu Gln | [SEQ ID NO.: 313] | | | |
| | 120 | 125 | | | |
| PEPTIDE #B52 Met-Asp-(15-125)hIL-3 pMON13476 | | | | | |
| 20 | Met Asp Asn Cys Ser Ile Met | Ile Asp Glu Ala Ile | His His Leu | | |
| | 15 | 20 | 25 | | |
| 25 | Lys Arg Pro Pro Ala Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ala | | |
| | 30 | 35 | 40 | | |
| | Glu Asp Val Asp Ile Leu Met | Glu Arg Asn Leu Arg | Leu Pro Asn | | |
| | 45 | 50 | 55 | | |
| 30 | Leu Glu Ser Phe Val Arg Ala | Val Lys Asn Leu Glu | Asn Ala Ser | | |
| | 60 | 65 | 70 | | |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | | |
| | 75 | 80 | 85 | | |
| 35 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | | |
| | 90 | 95 | 100 | | |
| 40 | Asp Trp Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | | |
| | 105 | 110 | 115 | | |
| | Leu Glu Gln Ala Gln Glu Gln | [SEQ ID NO.: 314] | | | |
| | 120 | 125 | | | |
| 45 | | | | | |
| PEPTIDE #B53 Met-Ala-(15-125)hIL-3 pMON13446 | | | | | |
| 50 | Met Ala Tyr Pro Glu Thr Asp Tyr Lys | Asp Asp Asp Asp Lys Asn | | | |
| | -14 | -10 | -5 | 15 | |
| | Cys Ser Ile Met Ile Asp Glu Ile Ile | His His Leu Lys Arg | Pro | | |
| | 20 | 25 | 30 | | |
| 55 | Pro Ala Pro Leu Leu Asp Pro Asn Asn | Leu Asn Ala Glu Asp | Val | | |
| | 35 | 40 | 45 | | |
| 60 | Asp Ile Leu Met Glu Arg Asn Leu Arg | Leu Pro Asn Leu Glu | Ser | | |
| | 50 | 55 | 60 | | |

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Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser Gly Ile Glu
 65 70 75
 5 Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser Ala Thr Ala
 80 85 90
 Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly Asp Trp Gln
 95 100 105
 10 Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr Leu Glu Gln
 110 115 120
 Ala Gln Glu Gln Gln [SEQ ID NO.: 315]
 125
 15

PEPTIDE #B54 Met-Ala-(15-125)hIL-3 pMON13390

20 Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys Asn
 -14 -10 -5 15
 Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu Lys Arg Pro
 20 25 30
 25 Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser Glu Asp Met
 35 40 45
 Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn Leu Leu Ala
 50 55 60
 30 Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser Gly Ile Glu
 65 70 75
 35 Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser Ala Thr Ala
 80 85 90
 Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly Asp Trp Gln
 95 100 105
 40 Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr Leu Glu Gln
 110 115 120
 Ala Gln Glu Gln Gln [SEQ ID NO.: 316]
 125
 45

PEPTIDE #C-2 Met-Ala-(15-125)hIL-3 pMON13400

50 Met Ala Asn Cys Ser Ile Met Pro Asp Glu Ala Ile His His Leu
 15 20 25
 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
 30 35 40
 55 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 60 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

140

| | | | | | | | |
|---|---------|---------------------|-----------------------|-------------|--|----|--|
| | | 75 | | 80 | | 85 | |
| | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | | | |
| | | 90 | 95 | 100 | | | |
| 5 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | | | |
| | | 105 | 110 | 115 | | | |
| 10 | Leu Glu | Gln Ala Gln Glu Gln | Gln [SEQ ID NO.: 317] | | | | |
| | | 120 | 125 | | | | |
| PEPTIDE #C-3 Met-Ala-(15-125)hIL-3 pMON13402 | | | | | | | |
| 15 | Met Ala | Asn Cys Ser Ile Met | Ile Asp Glu Leu Ile | His His Leu | | | |
| | | 15 | 20 | 25 | | | |
| | Lys Ile | Pro Pro Asn Pro Ser | Leu Asp Ser Ala Asn | Leu Asn Ser | | | |
| | | 30 | 35 | 40 | | | |
| 20 | Glu Asp | Val Ser Ile Leu Met | Glu Arg Asn Leu Arg | Thr Pro Asn | | | |
| | | 45 | 50 | 55 | | | |
| 25 | Leu Leu | Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser | | | |
| | | 60 | 65 | 70 | | | |
| | Gly Ile | Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | | | |
| | | 75 | 80 | 85 | | | |
| 30 | Ala Thr | Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly | | | |
| | | 90 | 95 | 100 | | | |
| | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | | | |
| | | 105 | 110 | 115 | | | |
| 35 | Leu Glu | Gln Ala Gln Glu Gln | Gln [SEQ ID NO.: 318] | | | | |
| | | 120 | 125 | | | | |
| PEPTIDE #C-10 Met-Ala-(15-125)hIL-3 pMON13440 | | | | | | | |
| 40 | Met Ala | Asn Cys Ser Ile Met | Ile Asp Glu Ala Ile | His His Leu | | | |
| | | 15 | 20 | 25 | | | |
| 45 | Lys Ile | Pro Pro Asn Pro Ser | Leu Asp Ser Ala Asn | Leu Asn Ser | | | |
| | | 30 | 35 | 40 | | | |
| | Glu Asp | Val Ser Ile Leu Met | Glu Arg Asn Leu Arg | Thr Pro Asn | | | |
| | | 45 | 50 | 55 | | | |
| 50 | Leu Leu | Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser | | | |
| | | 60 | 65 | 70 | | | |
| 55 | Gly Ile | Glu Pro Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser | | | |
| | | 75 | 80 | 85 | | | |
| | Ala Thr | Ala Ala Pro Ser Arg | Thr Pro Ile Ile Ile | Lys Ala Gly | | | |
| | | 90 | 95 | 100 | | | |
| 60 | Asp Trp | Gln Glu Phe Arg Glu | Lys Leu Thr Phe Tyr | Leu Val Thr | | | |

141

| | | | | | |
|---|---|--|-----|--|-----|
| | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 319] | | | | |
| 5 | 120 | | 125 | | |
| PEPTIDE #C-11 Met-Ala-(15-125)hIL-3 pMON13451 | | | | | |
| 10 | Met Ala Asn Cys Ser Ile Ile Leu Asp Glu Ala Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser | | | | |
| | 30 | | 35 | | 40 |
| 15 | Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| | Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser | | | | |
| 20 | 60 | | 65 | | 70 |
| | Gly Ile Glu Pro Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| | 75 | | 80 | | 85 |
| 25 | Ala Thr Ala Ala Pro Ser Arg Thr Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| | 105 | | 110 | | 115 |
| 30 | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 320] | | | | |
| | 120 | | 125 | | |
| PEPTIDE #C-4 Met-Ala-(15-125)hIL-3 pMON13403 | | | | | |
| 35 | Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu | | | | |
| | 15 | | 20 | | 25 |
| | Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser | | | | |
| 40 | 30 | | 35 | | 40 |
| | Glu Asp Met Asp Ile Leu Met Asp Ser Asn Leu Arg Thr Pro Asn | | | | |
| | 45 | | 50 | | 55 |
| 45 | Leu Leu Ala Phe Pro His Ala Ser Lys Gln Leu Glu Asn Ala Ser | | | | |
| | 60 | | 65 | | 70 |
| | Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser | | | | |
| | 75 | | 80 | | 85 |
| 50 | Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly | | | | |
| | 90 | | 95 | | 100 |
| | Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr | | | | |
| 55 | 105 | | 110 | | 115 |
| | Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 321] | | | | |
| | 120 | | 125 | | |
| 60 | PEPTIDE #C-5 Met-Ala-(15-125)hIL-3 pMON13411 | | | | |

142

5 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 10 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 15 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr
 105 110 115
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 322]
 120 125
 PEPTIDE #C-6 Met-Ala-(15-118)hIL-3 pMON13412
 30 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 35 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 40 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 45 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr Leu
 105 110 115 118
 50 [SEQ ID NO.: 323]
 PEPTIDE #C-7 Met-Ala-(15-125)hIL-3 pMON13413
 55 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 60 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

143

| | 45 | 50 | 55 |
|----|-----------------------------|---------------------|-------------|
| 5 | Leu Leu Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |
| | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| 10 | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| | Asp Trp Gln Glu Phe Arg Leu | Lys Leu Gln Phe Tyr | Leu Ser Ser |
| | 105 | 110 | 115 |
| 15 | Leu Glu Gln Ala Gln Glu Gln | [SEQ ID NO.: 324] | |
| | 120 | 125 | |

PEPTIDE #C-8 Met-Ala-(15-125)hIL-3 pMON13419

| | | | |
|----|-----------------------------|---------------------|-------------|
| 20 | Met Ala Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu |
| | 15 | 20 | 25 |
| 25 | Lys Arg Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ser |
| | 30 | 35 | 40 |
| | Glu Asp Met Asp Ile Leu Met | Asp Ser Asn Leu Leu | Thr Pro Asn |
| 30 | 45 | 50 | 55 |
| | Leu Leu Ala Phe Pro His Ala | Ser Lys Gln Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |
| 35 | Gly Ile Glu Ala Ile Leu Arg | Asn Leu Gln Pro Cys | Leu Pro Ser |
| | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Ser Arg | His Pro Ile Ile Ile | Lys Ala Gly |
| | 90 | 95 | 100 |
| 40 | Asp Trp Gln Glu Phe Arg Leu | Lys Leu Gln Phe Tyr | Leu Ser Ser |
| | 105 | 110 | 115 |
| | Leu Glu Gln Ala Gln Glu Gln | [SEQ ID NO.: 325] | |
| 45 | 120 | 125 | |

PEPTIDE #C-1 Met-Ala-(15-125)hIL-3 pMON13418

| | | | |
|----|-----------------------------|---------------------|-------------|
| 50 | Met Ala Asn Cys Ser Ile Met | Ile Asp Glu Ile Ile | His His Leu |
| | 15 | 20 | 25 |
| | Lys Arg Pro Pro Asn Pro Leu | Leu Asp Pro Asn Asn | Leu Asn Ser |
| | 30 | 35 | 40 |
| 55 | Glu Asp Met Asp Ile Leu Met | Glu Arg Asn Leu Arg | Thr Pro Asn |
| | 45 | 50 | 55 |
| 60 | Leu Leu Ala Phe Val Arg Ala | Val Lys His Leu Glu | Asn Ala Ser |
| | 60 | 65 | 70 |

144

Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
 75 85
 5 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 105 110 115
 10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 326]
 120 125

15 PEPTIDE #C-9 Met-Ala-(15-125)hIL-3 pMON13428

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 15 20 25
 20 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 30 35 40
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 55
 25 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 30 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
 75 80 85
 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly
 90 95 100
 35 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 327]
 120 125

40

PEPTIDE #C-12 Met-Ala-(15-125)hIL-3 pMON13459

45 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu
 15 20 25
 50 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
 30 35 40
 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 55 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
 75 80 85
 60 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly

145

90

95

100

5 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Thr
 105 110 115
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 328]
 120 125
 10 PEPTIDE #C-13 Met-Ala-(15-125)hIL-3 pMON13467
 15 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu
 15 20 25
 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
 30 35 40
 20 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 25 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 75 80 85
 30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 90 95 100
 Asp Trp Gln Glu Phe Arg Leu Lys Leu Gln Phe Tyr Leu Ser Ser
 105 110 115
 35 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 329]
 120 125
 40 PEPTIDE #C-14 Met-Ala-(15-125)hIL-3 pMON13492
 45 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Leu Ile His His Leu
 15 20 25
 Lys Ile Pro Pro Asn Pro Ser Leu Asp Ser Ala Asn Leu Asn Ser
 30 35 40
 50 Glu Asp Val Ser Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 45 50 55
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 60 65 70
 55 Gly Ile Glu Pro Ile Leu Ser Asn Leu Gln Pro Cys Val Pro Tyr
 75 80 85
 Trp Thr Ala Pro Pro Ser Arg Thr Pro Ile Thr Ile Lys Ala Gly
 90 95 100
 60 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

146

105

110

115

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO.: 330]
120 125

5

TABLE 4
DNA SEQUENCES

5 **pMON13287**

 Met-Ala- (15-125) IL-3

 DNA sequence #1
10 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
 G

 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
 A
15 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
 T

 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
20 G

 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
 G

25 GAACAACAG [SEQ ID NO:97]

 pMON13290

30 **Met-Ala- (15-125) IL-3**

 DNA sequence #2
 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
 G
35 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
 A

 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
40 T

 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
 G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
 G

 GAACAACAG [SEQ ID NO:98]

50

 pMON13313

55 **Met-Ala- (15-125) IL-3**

 DNA sequence #3
 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
 G
60 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
 A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G

10 GAACAACAG [SEQ ID NO:99]

pMON13288

15 **Met-Ala- (15-125) IL-3**

DNA sequence #4

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:100]

35

pMON13312

Met-Ala- (15-125) IL-3

40 **DNA sequence #5**

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO:101]

pMON13294

60 **Met-Ala- (15-125) IL-3**

DNA sequence #6

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G
5 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
10 T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:102]

20
pMONM13289

Met-Ala- (15-125) IL-3**DNA sequence #7**

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G
30 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
35 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
40 GAACAACAG [SEQ ID NO:103]

45
pMON13292

Met-Ala- (15-125) IL-3**DNA sequence #8**

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
50 G
CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A
55 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
60 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA

150

G

GAACAACAG [SEQ ID NO:104]

5

pMON13295

Met-Ala- (15-125) IL-3

10 DNA sequence #9

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G15 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
AAACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T20 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCTCTCGACATCCAATCACCATCAA
GGCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G

25

GAACAACAG [SEQ ID NO:105]

30

pMON13344

(15-125) IL-3

DNA sequence #10

35 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAAAATAACCTTCGTCGTCC
A40 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
TAAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

45

GACGGTGACTGGAATGAATTCCGTCTGTAAGTACCTTCTATCTGAAAACCTTGAGAACGCGCA
G

50

GCTCAACAG [SEQ ID NO:106]

pMON13345

55

(15-125) IL-3

DNA sequence #11

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCCTAACCTTTG

60 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

5 AAAAATCTCCTGCCATGTCTGCCCCGACGGCCGACCCACGCGACATCCAATCCATATCAA
G

GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

10 GCTCAACAG [SEQ ID NO:107]

pMON13346

15 (15-125) IL-3

DNA sequence #12

20 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG
CTGGACAGTAACAACCTCAATCCGAAGACATGGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

25 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCGACGGCCGACCCACGCGACATCCAATCCATATCAA
G

30 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:108]

35

pMON13347

40 (15-125) IL-3

DNA sequence #13

45 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCC
A

50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCGACGGCCGACCCACGCGACATCCAATCCATATCAA
G

55 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:109]

60

pMON13348

(15-125) IL-3

DNA sequence #14

5 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGACTTCC
A
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
AAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
15 G
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G
20 GCTCAACAG [SEQ ID NO:110]

pMON13349

25 (15-125) IL-3

DNA sequence #15

30 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
35 T
AAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G
40 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G
GCTCAACAG [SEQ ID NO:111]

45

pMON13350

(15-125) IL-3

50 DNA sequence #16

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
55 A
AACCTCGAGGCATCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
60 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGACCCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
G

5 GCTCAACAG [SEQ ID NO:112]

pMON13355

10 (15-125) IL-3

DNA sequence #17

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
15 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCTCC
A

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
20 T

CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCTCTCGACATCCAATCACCATCAA
G

GCAGGTGACTGGCAAGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
25 G

GCTCAACAG [SEQ ID NO:113]

30

pMON13352

(15-125) IL-3

35 **DNA sequence #18**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCTGCTCC
40 A

AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
45 G

GACGGTGACTGGAATGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50

GAACAACAG [SEQ ID NO:114]

pMON13354

55

(15-125) IL-3

DNA sequence #19

60 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG

154

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

5 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G

10 GACGGTGACTGGAATGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G

GAACAACAG [SEQ ID NO:115]

15 pMON13363
(15-125) IL-3 SECRETED

20 DNA sequence #20
AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTTG

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
25 A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T

30 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G

GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

35 GCTCAACAG [SEQ ID NO:116]

pMON13364

40 (15-125) IL-3 SECRETED

DNA sequence #21

45 AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTTG

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATATCAA
G

55 GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:117]

60

155

pMON13365**(15-125) IL-3 SECRETED****5 DNA sequence #22**

AACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTTG
CTGGACAGTAACAACCTCAATTCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
10 A
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
15 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G
GACGGTGACTGGAATGAATTCCGTCGTAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA
G
20 GCTCAACAG [SEQ ID NO:118]

pMON13360**25 (15-125) IL-3 SECRETED****DNA sequence #23**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
30 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
35 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
40 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO:119]
45

pMON13361**50 (15-125) IL-3 SECRETED****DNA sequence #24**

AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
55 CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
60 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA

156

G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
5 GAACAACAG [SEQ ID NO:120]

pMON13362
10 (15-125) IL-3 SECRETED
DNA sequence #25
15 AACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTG
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
20 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:121]
30

pMON13301
35 (15-125) IL-3 INTRACELLULAR
DNA sequence #26
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
40 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T
45 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G
GACGGTGAATGAATTCGTCGTAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
50 G
GCTCAACAG [SEQ ID NO:122]

pMON13302
55 (15-125) IL-3 INTRACELLULAR
DNA sequence #27
60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T

AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

10 GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
G

GCTCAACAG [SEQ ID NO:123]

15

pMON13303

(15-125) IL-3 INTRACELLULAR

20 **DNA sequence #28**
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTTCCACCTGCACCTTT
G

25 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGCAATTGAGAGCATTCT
T

30 AAAAATCTCCTGCCATGTCTGCCCCTGGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA
G

GACGGTGACTGGAATGAATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGAGAACGCGCA
G

35 GCTCAACAG [SEQ ID NO:124]

pMON13298

(15-125) IL-3 INTRACELLULAR

DNA sequence #29

45 ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
G

CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A

50 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCCTCTCGACATCCAATCATCATCAA
G

55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

60 GAACAACAG [SEQ ID NO:125]

pMON13299**(15-125) IL-3 INTRACELLULAR**

5 **DNA sequence #30**
ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
G
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
10 A
AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
15 CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
20 GAACAACAG [SEQ ID NO:126]

pMON13300

25 **Met-Ala- (15-125) IL-3 INTRACELLULAR**

DNA sequence #31
30 ATGGCTAACTGCTCTAACATGATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCT
G
CTGGACTTCAACAACCTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCC
A
35 AACCTCGAGGCATTCAACCGTGCTGTCAAGTCTCTGCAGAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCGTACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCACCATCAA
G
40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTTCCCTTGAGCACGCGCA
G
GAACAACAG [SEQ ID NO:127]
45 **DNA sequence #32**
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G
50 CTGGACCCGAACAACCTCAATTCTGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCT
T
55 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
60 G

159

GAACAACAG [SEQ ID NO: 160]

DNA sequence #33

5 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G
CTGGACCCGAACAACCTCAATTCTGAAGACATGGACATTTGATGGAACGAAACCTTCGAACTCCA
10 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAACGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO: 161]

20 DNA sequence #B1 pMON13406 Met-Ala-(15-125) IL-3
ATGGCAAAGTCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G
25 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
30 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
35 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 332]

40 DNA sequence #B2 pMON13414 Met-Ala-(15-125) IL-3
ATGGCAAAGTCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G
45 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
50 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 333]

60 DNA sequence #B3 pMON13407 Met-Ala-(15-125) IL-3

160

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

5 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 334]

20 DNA sequence #B4 pMON13405 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

25 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 335]

40 DNA sequence #B5 pMON13415 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAATGATCCATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

45 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 336]

60 DNA sequence #B6 pMON13408 Met-Ala-(15-125) IL-3

161

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

5 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATCGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 337]

DNA sequence #B7 pMON13409 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGCTGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 338]

DNA sequence #B8 pMON13410 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGGACGAACGAAACCTTCGAACTCC
A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 339]

DNA sequence #B9 pMON13422 Met-Ala-(15-125) IL-3

60 ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT

162

G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 340]

DNA sequence #B10 pMON13423 Met-Ala-(15-125) IL-3

20 ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGATCGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 341]

DNA sequence #B11 pMON13424 Met-Ala-(15-125) IL-3

40 ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACTCC
A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

50 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 342]

DNA sequence #B12 pMON13425 Met-Ala-(15-125) IL-3

60 ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

163

CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGCTGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 343]

15 DNA sequence #B13 pMON13426 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAGCTATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

20 CTGGACCCGAACAACCTCAATTCTGAAGACGTTGATATCCTGGACGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 344]

35 DNA sequence #B14 pMON13429 Met-Ala-(15-125) IL-3

ATGGCAAACCTGCTCTATAATCATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

40 CTGGACCCGAACAACCTCAATTCTGAAGACGTTATATCCTGGACGAACGAAACCTTCGAACTCCA
AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

50 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 345]

55 DNA sequence #B15 pMONM13368 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTT
G

60 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC

164

A

5 AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
10 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 346]

15 DNA sequence #B16 pMON13380 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTTCCACCTGCACCTTT
G
20 CTGGACAGTAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGCTCGCATTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
25 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
30 G
GAACAACAG [SEQ ID NO.: 347]

35 DNA sequence #B17 pMON13475 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G
40 CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
50 G
GAACAACAG [SEQ ID NO.: 348]

55 DNA sequence #B18 pMON13366 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATAACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
60 A

165

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO.: 349]

DNA sequence #B19 pMON13367 Met-Ala-(15-125)IL-3

15 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

30 GAACAACAG [SEQ ID NO.: 350]

DNA sequence #B20 pMON13369 Met-Ala-(15-125)IL-3 42D, 46S, 50D

35 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50 GAACAACAG [SEQ ID NO.: 351]

DNA sequence #B21 pMON13370 Met-Ala-(15-125)IL-3

55 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT

166

T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
5 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 352]
10
DNA sequence #B22 pMON13373 Met-Ala-(15-125) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
15 G
CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATTCTGATGGACCGAAACCTTCGACTTCC
A
20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
25 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 353]
30
DNA sequence #B23 pMON13374 Met-Ala-(15-125) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
35 G
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
45 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 354]
50
DNA sequence #B24 pMON13375 Met-Ala-(15-119) IL-3
ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
55 G
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

5 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAG [SEQ
ID NO.: 355]

DNA sequence #B25 pMON13376 Met-Asp-(15-119) IL-3

10 ATGGATAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

15 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 356]

25 DNA sequence #B26 pMON13377 Met-Ala-(15-119) IL-3

30 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTCCTCTATTCTGATGGACCGAAACCTTCGACTTCC
A

35 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

40 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 357]

45 DNA sequence #B27 pMON13378 Met-Asp-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

50 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

55 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

60 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 358]

168

DNA sequence #B28 pMON13379 Met-Ala-(15-125) IL-3

5 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATGCTGAAGACGTTTCTATCCTGATGGACCGAAACCTTCGACTTCC
A
10 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 359]
20

DNA sequence #B29 pMON13385 Met-Ala-(15-125) IL-3

25 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGGTACCACCTCGCCCTTC
C
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
30 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
35 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 360]
40

DNA sequence #B30 pMON13381 Met-Ala-(15-125) IL-3

45 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
50 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T
CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 361]
60

169

DNA sequence #B31 pMON13383 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G
5 CTGGACCCGAACAACCTCAATGACGAAGACGTTTCTATTCTGATGGACCGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
10 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
15 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 362]

20 DNA sequence #B32 pMON13384 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
25 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
30 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
35 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 363]

40 DNA sequence #B33 pMON13388 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G
45 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGACCGAAACCTTCGACTTAG
C
AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
50 T
CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G
55 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G
GAACAACAG [SEQ ID NO.: 364]

60 DNA sequence #B34 pMON13389 Met-Ala-(15-125) IL-3

170

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

5 CTGGACCCGAACAACCTCAATGACGAAGACATGGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 365]

20 DNA sequence #B35 pMON13391 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTC
C

25 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 366]

40 DNA sequence #B36 pMON13392 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

45 CTGGACCCGAACAACCTCAATGACGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 367]

60 DNA sequence #B37 pMON13393 Met-Ala-(15-125) IL-3

171

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C

5 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 368]

DNA sequence #B38 pMON13394 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

25 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 369]

DNA sequence #B39 pMON13395 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTC
C

CTGGACCCGAACAACCTCAATGACGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

45 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 370]

DNA sequence #B40 pMON13396 Met-Ala-(15-125) IL-3

60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT

172

G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

5

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCG
T

ATGGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO.: 371]

DNA sequence #B41 pMON13397 Met-Ala-(15-125) IL-3

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

25

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30

CGTAATCTCTGGCCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCCG
T

ATGGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO.: 372]

DNA sequence #B42 pMON13398 Met-Ala-(15-125) IL-3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

45

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

50

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO.: 373]

DNA sequence #B43 pMON13399 Met-Ala-(15-125) IL-3

60 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGGTACCACCTCGCCCTTC
C

173

CTGGACCCGAACAACCTCAATGACGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGACTTCC
A

5 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 374]

15 DNA sequence #B44 pMON13404 Met-Ala-(15-119) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

20 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

25 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGGTTACCCTTGAG [SEQ ID
NO.: 375]

35 DNA sequence #B45 pMON13387 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

40 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGACCGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50 GAACAACAG [SEQ ID NO.: 376]

55 DNA sequence #B46 pMON13416 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

60 CTGGACCCGAACAACCTCAATGACGAAGACGTCGATTCTCTGATGGAACGAAACCTTCGACTTCC
A

174

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO.: 377]

DNA sequence #B47 pMON13287 Met-Ala-(15-125) IL-3

15 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

CTGGACCCGAACAACCTCAATGACGAAGACGTCATGTCTCTGATGGAACGAAACCTTCGACTTCC
A

20 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

25 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

30 GAACAACAG [SEQ ID NO.: 378]

DNA sequence #B48 pMON13420 Met-Ala-(15-125) IL-3

35 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGACCTTCC
CTGGACCCGAACAACCTCAATGACGAAGACGTCCTATCCTGATGGACCGAAACCTTCACTTAGC

40 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

45 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

50 GAACAACAG [SEQ ID NO.: 379]

DNA sequence #B49 pMON13421 Met-Ala-(15-125) IL-3

55 ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C

CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTAG
C

60 AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

5 GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO.: 380]

10 DNA sequence #B50 pMON13432 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTC
C

15 CTGGACCCGAACAACCTCAATGACGAAGACATGTCTATCCTGATGGACCGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

20 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

25 GAACAACAG [SEQ ID NO.: 381]

30 DNA sequence #B51 pMON13382 Met-Ala-(15-125) IL-3

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTGCACCTTT
G

35 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

40 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAAACTGCAATTCTATCTGTGGACCCTTGAGCAAGCGCA
G

45 GAACAACAG [SEQ ID NO.: 382]

50 DNA sequence #B52 pMON13476 Met-Asp-(15-125) IL-3

ATGGATAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGAGACCACCTGCACCTTT
G

55 CTGGACCCGAACAACCTCAATGCTGAAGACGTCGATATCCTGATGGAACGAAACCTTCGACTTCC
A

AACCTGGAGAGCTTCGTAAGGGCTGTCAAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

60 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA

176

G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

5 GAACAACAG [SEQ ID NO.: 383]

pMON13400

10 Met-Ala-(15-125) IL-3

DNA sequence #C2

15 ATGGCTAACTGCTCTATAATGCCAGATGAAGCAATACATCACTTAAAGATACCACCTAACCCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

20 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

30 GAACAACAG [SEQ ID NO:384]

pMON13402

35 Met-Ala-(15-125) IL-3

DNA sequence #C3

40 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

45 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

50 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

55 GAACAACAG [SEQ ID NO:385]

pMON13440

60 Met-Ala-(15-125) IL-3

DNA sequence #C10

ATGGCTAACTGCTCTATTATGATCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCTAG
C

5 CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

10 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

15 GAACAACAG [SEQ ID NO:386]

20 pMON13451
Met-Ala-(15-125) IL-3
DNA sequence #C11

25 ATGGCTAACTGCTCTATAATACTCGATGAAGCAATACATCACTTAAAGATACCACCTAACCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

30 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

35 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

40 GAACAACAG [SEQ ID NO:387]

45 pMON13403
Met-Ala-(15-125) IL-3
DNA sequence #C4

50 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCC
A

55 AACCTGCTCGCATTCACACATGCTGTCAAGCAATTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

60

GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

5 GAACAACAG [SEQ ID NO:388]

pMON13419

Met-Ala-(15-125) IL-3

10 DNA sequence #C8

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

15 CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGACTCCAACCTTCGAACTCC
A

AACCTGCTCGCATTCACATGCTTCTAAGCAATTAGAAAATGCATCAGGTATTGAGGCAATTCT
20 T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

25 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:389]

30 pMON13411

Met-Ala-(15-125) IL-3

DNA sequence #C5

35 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
40 A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

45 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
50 G

GAACAACAG [SEQ ID NO:390]

pMON13412

55 Met-Ala-(15-118) IL-3

DNA sequence #C6

60 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

5 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

10 GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTTAATA
[SEQ ID NO:391]

15 pMON13413
Met-Ala-(15-125) IL-3
DNA sequence #C7

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

30 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCA
G

35 GAACAACAG [SEQ ID NO:392]

40 pMON13418
Met-Ala-(15-125) IL-3
DNA sequence #C1

45 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCTTT
G

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

50 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCTCCCTCTCGAACACCAATCAGATCAA
G

55 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

60 GAACAACAG [SEQ ID NO:393]

pMON13428

Met-Ala-(15-125) IL-3

5

DNA sequence #C9

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGAGACCACCTAACCCCTTT
G

10

CTGGACCCGAACAACCTCAATTCCGAAGACATGGATATCCTGATGGAACGAAACCTTCGAACTCC
A

15

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

20

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:394]

25

pMON13459

Met-Ala-(15-125) IL-3

30

DNA sequence #C12

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAG
C

35

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

40

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

45

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:395]

50

pMON13467

Met-Ala-(15-125) IL-3

55

DNA sequence #C13

ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCCTAG
C

60

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

181

AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCT
T

5 CGTAATCTCCAACCATGTCTGCCCTCTGCCACGGCCGCACCCTCTCGACATCCAATCATCATCAA
G

GCAGGTGACTGGCAAGAATTCCGGCTTAACTGCAATTCTATCTGTCTTCTCTTGAGCAAGCGCA
G

10 GAACAACAG [SEQ ID NO:396]

pMON13492

15 Met-Ala-(15-125) IL-3

DNA sequence #C14

20 ATGGCTAACTGCTCTATAATGATCGATGAAATTATACATCACTTAAAGATACCACCTAACCCTAG
C

CTGGACAGTGCTAACCTCAATTCCGAAGACGTCTCTATCCTGATGGAACGAAACCTTCGAACTCC
A

25 AACCTGCTCGCATTCGTAAGGGCTGTCAAGCACTTAGAAAATGCATCAGGTATTGAGCCAATTCT
T

TCTAATCTCCAACCATGTGTTCCCTATTGGACGGCCCCCTCCCTCTCGAACACCAATCACGATCAA
G

30 GCAGGTGACTGGCAAGAATTCCGGGAAAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCA
G

GAACAACAG [SEQ ID NO:397]

35 pMON13446

40 Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-Ala (15-
125) IL-3

DNA sequence #B53

45 ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGA
T

GAAATTATACATCACTTAAAGAGACCACCTGCACCTTTGCTGGACCCGAACAACCTCAATGCTGA
A

50 GACGTCGATATCCTGATGGAACGAAACCTTCGACTTCCAAACCTGGAGAGCTTCGTAAGGGCTGT
C

AAGAACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTC
T

55 GCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGA
A

AAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:404]

60

182

pMON13390

Met-Ala-Tyr-Pro-Glu-Thr-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-Ala (15-125) IL-3

5

DNA sequence #B54

ATGGCATATCCAGAACTGATTACAAGGACGACGATGACAAGGCTAACTGCTCTATAATGATCGA
T
10 GAAATTATACATCACTTAAAGAGACCACCTAACCCTTTGCTGGACCCGAACAACCTCAATTCCGA
A
GACATGGATATCCTGATGGAACGAAACCTTCGAACTCCAAACCTGCTCGCATTCTGTAAGGGCTGT
15 C
AAGCACTTAGAAAATGCATCAGGTATTGAGGCAATTCTTCGTAATCTCCAACCATGTCTGCCCTC
T
20 GCCACGGCCGCACCCTCTCGACATCCAATCATCATCAAGGCAGGTGACTGGCAAGAATTCCGGGA
A
AAACTGACGTTCTATCTGGTTACCCTTGAGCAAGCGCAGGAACAACAG [SEQ ID NO:405]

25

Polypeptides corresponding to [SEQ ID NO. 129] comprising (1-133)hIL-3 containing four or more amino acid substitutions can be made using the procedures described above and in the following examples by starting with the appropriate oligonucleotides and then constructing the DNA encoding the polypeptide and expressing it in an appropriate host cell. In a similar manner polypeptides which correspond to [SEQ ID NO. 130] and contain four or more amino acid substitutions and wherein from 1 to 14 amino acids have been sequentially deleted from the N-terminus, or from 1 to 15 amino acids have been deleted from the C-terminus or deletions of amino acids have been made from both the N-terminus and the C-terminus can also be made by following the procedures described above and in the following examples, beginning with the appropriate starting materials.

Further details known to those skilled in the art may be found in T. Maniatis, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor

Laboratory (1982) and references cited therein,
 incorporated herein by reference; and in J. Sambrook,
 et al., Molecular Cloning, A Laboratory Manual, 2nd
 edition, Cold Spring Harbor Laboratory (1989) and
 5 references cited therein, incorporated herein by
 reference.

The following examples will illustrate the
 invention in greater detail although it will be
 understood that the invention is not limited to these
 10 specific examples.

Amino acids are shown herein by standard one
 letter or three letter abbreviations as follows:

| | | | |
|----|-------------------------|-----|---------------|
| 15 | Abbreviated Designation | | Amino Acid |
| | A | Ala | Alanine |
| | C | Cys | Cysteine |
| 20 | D | Asp | Aspartic acid |
| | E | Glu | Glutamic acid |
| | F | Phe | Phenylalanine |
| | Abbreviated Designation | | Amino Acid |
| 25 | G | Gly | Glycine |
| | H | His | Histidine |
| | I | Ile | Isoleucine |
| | K | Lys | Lysine |
| 30 | L | Leu | Leucine |
| | M | Met | Methionine |
| | N | Asn | Asparagine |
| | P | Pro | Proline |
| | Q | Gln | Glutamine |
| 35 | R | Arg | Arginine |
| | S | Ser | Serine |
| | T | Thr | Threonine |
| | V | Val | Valine |

| | | |
|---|-----|------------|
| W | Trp | Tryptophan |
| Y | Tyr | Tyrosine |

5 Various other examples will be apparent to the
person skilled in the art after reading the present
disclosure without departing from the spirit and scope
of the invention. It is intended that all such other
examples be included within the scope of the appended
claims.

10

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10 EXAMPLE 1

Construction of pMON 5846 (Fig. 4) which encodes [Met-
(1-133)hIL-3 (Arg¹²⁹)]

- A plasmid containing the gene for the cDNA of
hIL-3 cloned into pUC18 on an EcoRI to HindIII
15 fragment was obtained from British Biotechnology
Limited (Cambridge, England). This plasmid was
designated pPO518. The purified plasmid DNA was
cleaved by the restriction endonucleases NheI and
BamHI. Approximately 0.5 micrograms of cleaved
20 plasmid DNA was ligated to 1.0 picomoles of a pair of
annealed oligonucleotides with the following sequence:

5'-CTAGCGATCTTTTAATAAGCTTG-3' [SEQ ID NO: 1]

3'-GCTAGAAAATTATTCGAACCTAG-5' [SEQ ID NO: 2]

25

- The ligation mixture was used to transform
competent JM101 cells to ampicillin resistance.
Colonies were picked, and plasmid DNA was purified and
subjected to restriction enzyme analysis. An isolate
30 was identified in which the above oligonucleotide
sequence had replaced the portion of the gene that
encodes the extreme C terminus. Within the new
sequence was a new stop codon, TAA, and a recognition
site for the enzyme HindIII. The new plasmid was
35 designated pMON5846.

EXAMPLE 2(a) Construction of expression vector plasmid pMON2341

5 The plasmid pMON2341 was used to supply the particular replicon and expression elements used for construction of many of the plasmids used to produce hIL-3 and hIL-3 muteins in E. coli. These expression elements are described in the materials and methods
10 section. pMON2341 is derived from pMON5515 (Olins et al., 1988) and from pMON2429. pMON2429 consists of the phage mp18 (Yanisch-Perron et al., 1985) with a BclI fragment carrying the chloramphenicol acetyl transferase (cat) gene from pBR328 (Covarrubias et
15 al., 1981) inserted into the BamHI site. The cat gene in pMON2429 has been altered from that in pBR328 by site directed mutagenesis (Kunkel, 1985). The recognition sites for NcoI and EcoRI which occur in the native gene were altered so that these two
20 restriction enzymes no longer recognize these sites. The changes did not alter the protein specified by the gene. Also, an NcoI site was introduced at the N-terminus of the coding sequence so that it overlaps the codon for initiator methionine.

25 The steps involved in construction of pMON2341 are listed below:

(1) The DNAs of pMON5515 and pMON2429 were treated with NcoI and HindIII. The fragments were ligated and used to transform competent E. coli to
30 ampicillin resistance. From these colonies, some were identified that were chloramphenicol resistant. From one of these colonies, plasmid DNA was isolated in which the rat atriopeptigen gene of pMON5515 had been replaced by the NcoI to HindIII fragment containing
35 the cat gene from pMON2429. This fragment contains the recognition sites for several restriction enzymes

in the portion derived from the multilinker region of mp18. The new plasmid was designated pMON2412.

(2) pMON2412 was treated with the enzyme ClaI which cleaves at one location in the pBR327 derived
5 portion of the DNA. The protruding ends were rendered blunt by treatment with Klenow in the presence of nucleotide precursors. This DNA was mixed with an isolated 514 bp RsaI fragment derived from pEMBL8 (Dente et al., 1983). This RsaI fragment contains the
10 origin of replication of phage f1. This ligation mixture was used to transform competent E. coli cells to ampicillin resistance. Among the plasmid DNAs isolated from these cells was pMON5578. This plasmid has the structure of pMON2412 with the f1 origin
15 region inserted into the ClaI site. This is illustrated in the Figures and in Olins and Rangwala (1990).

(3) The DNA of pMON5578 was treated with restriction enzymes HindIII and MstII. The DNA was
20 then treated with Klenow enzyme in the presence of nucleotide precursors to render the ends blunt. This treated DNA was ligated and used to transform competent E. coli to ampicillin resistance. From the ampicillin resistant colonies, one plasmid was
25 recovered from which the portion between HindIII and MstII was absent. This deletion resulted in the removal of sequences from the plasmid which are recognized by a number of restriction endonuclease sites. The new plasmid was designated pMON5582.

30 (4) The DNA of pMON5582 was treated with SstII and BclII and ligated in the presence of annealed oligonucleotides with the sequences shown below.

5'- GGCAACAATTTCTACAAAACACTTGATACTGTATGAGCAT-
35 3'-CGCCGTTGTTAAAGATGTTTTGTGAACATATGACATACTCGTA-

ACAGTATAATTGCTTCAACAGAACAGATC-3' [SEQ ID NO:3]

TGTCATATTAAACGAAGTTGTCTTGT-5' [SEQ ID NO:4]

This sequence encodes the essential elements of
5 the *recA* promoter of *E. coli* including the
transcription start site and the *lexA* repressor
binding site (the operator) (Sancar et al., 1980).
The plasmid recovered from the ligation mixes
contained this *recA* promoter in place of the one in
10 pMON5582 (and in pMON5515). The functionality of the
recA promoter was illustrated by Olins and Rangwala
(1990). The new plasmid was designated pMON5594.

(5) To eliminate the single *EcoRI* site in
pMON5594, the DNA was treated with *EcoRI*, then with
15 Klenow in the presence of nucleotide precursors to
render the ends blunt and then the DNA was ligated.
From this ligation mix a plasmid was recovered whose
DNA was not cleaved with *EcoRI*. This plasmid was
designated pMON5630.

20 (6) To alter the single recognition site for
PstI, plasmid pMON5630 was subjected to site directed
mutagenesis (Kunkel, 1985). The oligonucleotide used
in this procedure has the sequence shown below.

25 5'-CCATTGCTGCCGGCATCGTGGTC-3' [SEQ ID NO:5]

The result of the procedure was to construct
pMON2341 which differs from pMON5630 in that the *PstI*
site in the beta-lactamase gene was altered so that
30 *PstI* no longer recognizes the site. The single
nucleotide change does not alter the amino acid
sequence of the beta-lactamase protein.

(b) Construction of pMON5847 (Fig. 5) which encodes
[Met-(1-133)hIL-3(Arg¹²⁹)]

35 Plasmid pMON2341 was used to supply the replicon,
promotor, ribosome binding site, transcription

terminator and antibiotic resistance marker for the plasmids used to produce hIL-3 in E. coli from cDNA derived hIL-3 genes.

Plasmid pMON2341 was treated with restriction
5 enzymes NcoI and HindIII. The restriction fragment containing the replication origin was purified. The DNA of plasmid pMON5846 was treated with NcoI and HindIII. The restriction fragment containing the hIL-3 gene was gel purified. These purified restriction
10 fragments were mixed and ligated. The ligation mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked, and plasmid DNA was purified and analyzed using restriction enzymes. pMON5847 was identified as a
15 plasmid with the replicon of pMON2341 and the hIL-3 gene in place of the chloramphenicol acetyl transferase gene. JM101 cells harboring this plasmid were cultured in M9 medium and treated with nalidixic acid as described above. Samples of the culture were
20 examined for protein content. It was found that this hIL-3 mutein was produced at about 6% of total cell protein as measured on Coomassie stained polyacrylamide gels.

25

EXAMPLE 3

Construction of pMON5854 (Fig. 7) which encodes [Met-(1-133)hIL-3(Arg¹²⁹)]

To increase the accumulation of hIL-3 in E. coli, the coding sequence of the amino terminal portion of
30 the protein was altered to more closely reflect the codon bias found in E. coli genes that produce high levels of proteins (Gouy and Gautier, 1982). To change the coding sequence for the amino terminal portion of the gene, a pair of synthetic
35 oligonucleotides were inserted between the NcoI and HpaI sites within the coding sequence. About 0.5

micrograms of DNA of the plasmid pMON5847 (Example 2) was treated with NcoI and HpaI. This DNA was mixed with an annealed pair of oligonucleotides with the following sequence:

5

5'-CATGGCTCCAATGACTCAGACTACTTCTCTTAAGACT-
3'-CGAGGTTACTGAGTCTGATGAAGAGAATTCTGA-

TCTTGGGTT-3' [SEQ ID NO:6]

10 AGAACCCAA-5' [SEQ ID NO:7]

The fragments were ligated. The ligation mixture was used to transform competent JM101 to ampicillin resistance. Colonies were picked into broth. From the cultures plasmid DNA was made and examined for the presence of a DdeI site (CTNAG) which occurs in the synthetic sequence but not between the NcoI and HpaI sites in the sequence of pMON5847. The new recombinant plasmid was designated pMON5854. The nucleotide sequence of the DNA in the coding sequence of the amino terminal portion of the hIL-3 gene in pMON5854 was determined by DNA sequencing and found to be the same as that of the synthetic oligonucleotide used in ligation. Cultures of JM101 cells harboring this plasmid were grown and treated with nalidixic acid to induce production of the hIL-3 mutant protein. Analysis of the proteins on Coomassie gels showed that the accumulation of hIL-3 mutein was about 25% of total cell protein in cultures harboring pMON5854, significantly higher than it was in cultures harboring pMON5847.

EXAMPLE 4

Construction of pMON5887 (Fig. 12) which encodes [Met-(1-125)hIL-3]

35

The plasmid DNA of pMON5854 (Example 3) was

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treated with EcoRI and HindIII and the larger fragment gel was purified. About 0.5 microgram of this DNA was ligated to 1 picomole of an annealed pair of oligonucleotides which encode amino acids 107 through
 5 125 of hIL-3. The sequences of these oligonucleotides are shown below.

EcoRI to HindIII

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAA-
 10 3'-GGCAGCATTTGACTGGAAGATAGACTTTT-

CCTTGGAGAACGCGCAGGCTCAACAGTAATA-3' [SEQ ID NO:8]

GGAACCTCTTGCGCGTCCGAGTTGTCATTATTCGA-5' [SEQ ID NO:9]

15 After ligation, the DNA was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and plasmid DNA was isolated from each culture. Restriction analysis of the plasmid DNA showed the presence of an EcoRI to
 20 HindIII fragment smaller than that of pMON5854. The nucleotide sequence of the portion of the coding sequence between the EcoRI and HindIII sites was determined to confirm the accuracy of the replaced sequence. The new plasmid was designated pMON5887
 25 encoding Met-(1-125)hIL-3 which has the following amino acid sequence:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
 Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 30 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 35 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:10]

EXAMPLE 5

Construction of pMON5967 which encodes [Met-Ala-(15-125)hIL-3]

5 Plasmid DNA of pMON5887 isolated from E. coli GM48 (dam-) was cleaved with NcoI and ClaI and ligated to 1 picomole of an annealed pair of oligonucleotides, encoding amino acids [Met Ala (15-20)hIL-3]. The
10 sequence of these oligonucleotides is shown below.
5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:11]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:12]

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant
15 colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be smaller than that of pMON5887 by restriction analysis using NcoI and NsiI. The nucleotide sequence of the region between NcoI and ClaI was determined and
20 found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5967 and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein purification and bio-assay.

25

EXAMPLE 6

Construction of pMON5978 which encodes [Met-Ala-(15-125)hIL-3]

30 Plasmid DNA of pMON5967 isolated from E. coli GM48(dam-) was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six oligonucleotides encoding hIL-3 amino acids 20-70 (FIG. 2). This synthetic fragment encodes three
35 unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in

Figure 2.

The resulting ligation mix was used to transform competent *E. coli* JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated and screened with XbaI and EcoRV for the presence of the new restriction site EcoRV. The DNA sequence of the region between ClaI and NsiI was determined and found to be the same as that of the synthetic oligonucleotides. The new plasmid was designated pMON5978, and cells containing it were induced for protein production. Sonicated cell pellets and supernatants were used for protein purification and bio-assay.

Plasmid pMON5978 encodes [Met-Ala-(15-125)hIL-3] which has the following amino acid sequence:

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:13]

25

EXAMPLE 7

Construction of pMON13356

Plasmid pMON5988 DNA was digested with restriction enzymes NcoI and EcoRV, and the resulting 4190 base pair NcoI,EcoRV fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 47-125 of (15-125)hIL-3. The 4190 base pair NcoI,EcoRV restriction fragment

from pMON5988 was ligated to the following annealed complementary oligonucleotides from Table (2).

Oligo #13 [SEQ ID NO:27]

5 **Oligo #14** [SEQ ID NO:28]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB
10 broth and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 99 bases between the NcoI and EcoRV restriction sites in the (15-125) hIL-3 gene
15 are replaced with 22 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

20

EXAMPLE 8

Construction of pMON13344

Plasmid pMON13356 DNA was digested with restriction enzymes NcoI and EcoRV, and the resulting 4190 base pair NcoI,EcoRV fragment contains the following
25 genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 47-125 of (15-125)hIL-
30 3. The second DNA fragment was generated by synthetic gene assembly using the following complementary oligonucleotide pairs that have overlapping ends:

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Oligo #1 [SEQ ID NO:15]**Oligo #2** [SEQ ID NO:16]**Oligo #3** [SEQ ID NO:17]5 **Oligo #4** [SEQ ID NO:18]**Oligo #9** [SEQ ID NO:23]**Oligo #10** [SEQ ID NO:24]

The assembled oligonucleotides create NcoI and EcoRV
10 restriction ends and the DNA sequence that encodes
amino acids 15-46 of (15-125)hIL-3 with the following
amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A
and 45V. The codons encoding amino acids 15-46 of
(15-125)hIL-3 are those found in the hIL-3 cDNA
15 sequence except at those positions where amino acid
substitutions were made. The 4190 base pair
NcoI,EcoRV restriction fragment from pMON13356 was
ligated with the pairs of annealed oligonucleotides.
The ligation reaction was digested with NdeI and
20 subsequently used to transform E. coli K-12 strain
JM101. Transformant bacteria were selected on
ampicillin-containing plates. Plasmid DNA was
isolated from a colony grown in LB broth. The DNA
sequence was determined to be that of the
25 oligonucleotides. The plasmid, pMON13344, encodes the
(15-125)hIL-3 variant with the following amino acid
sequence:

Peptide #2

30 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
35 Glu Asp Val Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
40 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

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Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
5 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
10 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:66]

DNA sequence #10 [SEQ ID NO:106] codes for the
foregoing pMON13344 polypeptide.

EXAMPLE 9

15 Construction of pMON13345

The 4190 base pair NcoI, EcoRV restriction fragment
from pMON13356 was ligated with the following pairs of
annealed complementary oligonucleotides:

Oligo #1 [SEQ ID NO:15]
20 **Oligo #2** [SEQ ID NO:16]

Oligo #5 [SEQ ID NO:19]
Oligo #6 [SEQ ID NO:20]

25 **Oligo #11** [SEQ ID NO:25]
Oligo #12 [SEQ ID NO:26]

The assembled oligonucleotides create NcoI and EcoRV
restriction ends and the DNA sequence that encodes
30 amino acids 15-46 of (15-125)hIL-3 with the following
amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S
and 45M. The codons encoding amino acids 15-46 of
(15-125)hIL-3 are those found in the hIL-3 cDNA
sequence except at those positions where amino acid
35 substitutions were made. The ligation reaction was
digested with NdeI and used to transform E. coli K-12
strain JM101. Transformant bacteria were selected on
ampicillin-containing plates. Plasmid DNA was
isolated from a colony grown in LB broth. The DNA was
40 sequenced to determine that the sequence was that of

204

the oligonucleotides. The plasmid, pMON13345, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #3

5 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
10 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
15 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
20 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:67]

DNA sequence #11 [SEQ ID NO:107] codes for the foregoing pMON13345 polypeptide.

30

EXAMPLE 10Construction of pMON13346

The 4190 base pair NcoI, EcoRV restriction fragment from pMON13356 was ligated with the following pairs of
35 annealed complementary oligonucleotides:

Oligo #1 [SEQ ID NO:15]**Oligo #2** [SEQ ID NO:16]**Oligo #7** [SEQ ID NO:21]40 **Oligo #8** [SEQ ID NO:22]**Oligo #11** [SEQ ID NO:25]**Oligo #12** [SEQ ID NO:26]

45 The assembled oligonucleotides create NcoI and EcoRV

restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125)hIL-3 with the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S and 45M. The codons encoding amino acids 15-46 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and DNA sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13346, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #4

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:68]

DNA sequence #12 [SEQ ID NO:108] codes for the foregoing pMON13346 polypeptide.

EXAMPLE 11**Construction of pMON13357**

Plasmid pMON5988 DNA was digested with restriction

enzymes EcoRV and NsiI, and the resulting 4218 base pair EcoRV,NsiI fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication
5 as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. The 4218 base pair EcoRV,NsiI restriction fragment from pMON5988 was ligated to the
10 following annealed complementary oligonucleotides:

Oligo #19 [SEQ ID NO:33]

Oligo #20 [SEQ ID NO:34]

15 The ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by
20 restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 71 bases between the EcoRV and NsiI restriction sites in the (15-125)hIL-3 gene are replaced with 22 bases from the above mentioned
25 oligonucleotides. This linker also contains a NdeI recognition sequence.

EXAMPLE 12

Construction of pMON13347

30 The 4218 base pair EcoRV,NsiI restriction fragment from pMON13357 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #21 [SEQ ID NO:35]

35 **Oligo #22** [SEQ ID NO:36]

Oligo #25 [SEQ ID NO:39]

Oligo #26 [SEQ ID NO:40]

Oligo #31 [SEQ ID NO:45]

5 **Oligo #32** [SEQ ID NO:46]

The assembled oligonucleotides create EcoRV and NsiI restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55L, 59L, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13347, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #5

25 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
30 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
35 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
40 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
45 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:69]

DNA sequence #13 [SEQ ID NO:109] codes for the foregoing pMON13347 polypeptide.

EXAMPLE 13

5 Construction of pMON13348

The 4218 base pair EcoRV, NsiI restriction fragment from pMON13357 was ligated with the following pairs of annealed complementary oligonucleotides:

10 **Oligo #21** [SEQ ID NO:35]

Oligo #22 [SEQ ID NO:36]

Oligo #27 [SEQ ID NO:41]

Oligo #28 [SEQ ID NO:42]

15

Oligo #31 [SEQ ID NO:45]

Oligo #32 [SEQ ID NO:46]

The assembled oligonucleotides create EcoRV and NsiI
20 restriction ends and the DNA sequence that encodes amino acids 47-71 of (15-125)hIL-3 with the following amino acid substitutions: 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence
25 except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated
30 from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13348, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

35 **Peptide #6**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

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Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 5 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 10 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 20 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:70]

DNA sequence #14 [SEQ ID NO:110] encodes the foregoing pMON13348 polypeptide.

25

EXAMPLE 14

Construction of pMON13349

The 4218 base pair EcoRV, NsiI restriction fragment from
 pMON13357 was ligated with the following pairs of
 30 annealed complementary oligonucleotides:

Oligo #23 [SEQ ID NO:37]

Oligo #24 [SEQ ID NO:38]

35 **Oligo #25** [SEQ ID NO:39]

Oligo #26 [SEQ ID NO:40]

Oligo #29 [SEQ ID NO:43]

Oligo #30 [SEQ ID NO:44]

40

The assembled oligonucleotides create EcoRV and NsiI
 restriction ends and the DNA sequence that encodes
 amino acids 47-71 of (15-125)hIL-3 with the following
 amino acid substitutions: 51R, 55T, 59L, 62V, 67H and

69E. The codons encoding amino acids 47-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was
 5 digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth and the DNA was
 10 sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13349, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #7

15 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 20 Glu Asp Gln Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 25 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 30 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 35 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:71]

DNA sequence #15 [SEQ ID NO:111] encodes the foregoing pMON13349 polypeptide.

40 EXAMPLE 15

Construction of pMON13358

Plasmid pMON5988 DNA was digested with restriction enzymes NsiI and EcoRI and the resulting 4178 base pair NsiI,EcoRI fragment contains the following genetic
 45 elements: beta-lactamase gene (AMP), pBR327 origin of

replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-71 and 106-125 of (15-125)hIL-3. The 4178 base pair NsiI,EcoRI restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides.

Oligo #15 [SEQ ID NO:29]

10 **Oligo #16** [SEQ ID NO:30]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 111 bases between the NsiI and EcoRI restriction sites in the (15-125) hIL-3 gene are replaced with 24 bases from the above mentioned oligonucleotides. This linker also contains a NdeI recognition sequence.

25 EXAMPLE 16

Construction of pMON13350

The 4178 base pair NsiI,EcoRI restriction fragment from pMON13358 was ligated with the following pairs of annealed complementary oligonucleotides:

30

Oligo #41 [SEQ ID NO:55]

Oligo #42 [SEQ ID NO:56]

Oligo #39 [SEQ ID NO:53]

35 **Oligo #40** [SEQ ID NO:54]

212

Oligo #35 [SEQ ID NO:49]

Oligo #36 [SEQ ID NO:50]

Oligo #43 [SEQ ID NO:57]

5 Oligo #44 [SEQ ID NO:58]

The assembled oligonucleotides create NsiI and EcoRI restriction ends and the DNA sequence that encodes amino acids 72-105 of (15-125)hIL-3 with the following amino acid substitutions: 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A and 105Q. The codons encoding amino acids 72-105 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13350, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #8

25 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 30 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 35 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 40 Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 45 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:72]

DNA sequence #16 [SEQ ID NO:112] codes for the foregoing pMON13350 polypeptide.

5

EXAMPLE 17

Construction of pMON13355

The 4178 base pair NsiI, EcoRI restriction fragment from pMON13358 was ligated with the following pairs of
10 annealed complementary oligonucleotides:

Oligo #41 [SEQ ID NO:55]

Oligo #42 [SEQ ID NO:56]

15

Oligo #37 [SEQ ID NO:51]

Oligo #38 [SEQ ID NO:52]

Oligo #33 [SEQ ID NO:47]

Oligo #34 [SEQ ID NO:48]

20

Oligo #43 [SEQ ID NO:57]

Oligo #44 [SEQ ID NO:58]

The assembled oligonucleotides create NsiI and EcoRI
25 restriction ends and the DNA sequence that encodes amino acids 72-105 of (15-125)hIL-3 with the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A and 105Q. The codons encoding amino acids 72-105 of (15-125)hIL-3 are those found in the hIL-3
30 cDNA sequence except at those positions where amino acid substitutions were made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was
35 isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of

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the oligonucleotides. The plasmid, pMON13355, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

5 **Peptide #9**
 Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 10 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 25 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:73]

DNA sequence #17 [SEQ ID NO:113] codes for the foregoing pMON13355 polypeptide.
 30

EXAMPLE 18

Construction of pMON13359

Plasmid pMON5988 DNA was digested with restriction
 35 enzymes EcoRI and HindIII, and the resulting 4225 base pair EcoRI,HindIII fragment contains the following genetic elements: beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, g10L
 40 ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-105 of (15-125)hIL-3. The 4225 base pair EcoRI,HindIII restriction fragment from pMON5988 was ligated to the following annealed complementary oligonucleotides.

45

Oligo #17 [SEQ ID NO:31]

Oligo #18 [SEQ ID NO:32]

5 The ligation reaction was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth, and the size of the inserted fragment was determined by restriction
10 analysis employing restriction enzymes NcoI and HindIII in double digest. In the resulting plasmid the 64 bases between the EcoRI and HindIII restriction sites in the (15-125)hIL-3 gene are replaced with 20 bases from the above mentioned oligonucleotides. This linker
15 also contains an NdeI recognition sequence.

EXAMPLE 19

Construction of pMON13352

20 The 4225 base pair EcoRI,HindIII restriction fragment from pMON13359 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #45 [SEQ ID NO:59]

25 **Oligo #46** [SEQ ID NO:60]

Oligo #49 [SEQ ID NO:63]

Oligo #50 [SEQ ID NO:64]

30 The assembled oligonucleotides create EcoRI and HindIII restriction ends and the DNA sequence that encodes amino acids 106-125 of (15-125)hIL-3 with the following amino acid substitutions: 109E, 116V, 120Q and 123E. The codons encoding amino acids 106-125 of (15-125)hIL-
35 3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were

made. The ligation reaction was digested with NdeI and used to transform E. coli K-12 strain JM101.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated from a colony grown in LB broth. The DNA was sequenced to determine that the sequence was that of the oligonucleotides. The plasmid, pMON13352, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #10**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

15 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

20 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

25 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

30 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:74]

DNA sequence #18 [SEQ ID NO:114] codes for the foregoing pMON13352 polypeptide.

35

EXAMPLE 20

Construction of pMON13354

The 4225 base pair EcoRI, HindIII restriction fragment from pMON13359 was ligated with the following pairs of annealed complementary oligonucleotides:

Oligo #45 [SEQ ID NO:59]

Oligo #46 [SEQ ID NO:60]

45

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Oligo #47 [SEQ ID NO:61]

Oligo #48 [SEQ ID NO:62]

The assembled oligonucleotides create EcoRI and HindIII
 5 restriction ends and the DNA sequence that encodes
 amino acids 106-125 of (15-125)hIL-3 with the following
 amino acid substitutions: 109E, 116V, 117S, 120H and
 123E. The codons encoding amino acids 106-125 of (15-
 10 125)hIL-3 are those found in the hIL-3 cDNA sequence
 except at those positions where amino acid
 substitutions were made. The ligation reaction was
 digested with NdeI and used to transform E. coli K-12
 strain JM101. Transformant bacteria were selected on
 ampicillin-containing plates. Plasmid DNA was isolated
 15 from a colony grown in LB broth, and the DNA was
 sequenced to determine that the sequence was that of
 the oligonucleotides. The plasmid, pMON13354, encodes
 the (15-125)hIL-3 variant with the following amino acid
 sequence:

20 **Peptide #11**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 25

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 30

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 35

Asp Trp Asn Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 40

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:75]

DNA sequence #19 [SEQ ID NO:115] codes for the
 45 foregoing pMON13354 polypeptide.

EXAMPLE 21Construction of pMON13360

5 Plasmid pMON13352 DNA was digested with restriction
enzymes NsiI and EcoRI, resulting in a 4178 base pair
NsiI,EcoRI fragment. The genetic elements derived from
pMON13352 are the beta-lactamase gene (AMP), pBR327
10 origin of replication, phage f1 origin of replication
as the transcription terminator, pAraBAD promoter, g10L
ribosome binding site, lamB secretion leader and the
bases encoding amino acids 15-71 and 106-125 of (15-
125)hIL-3. Plasmid pMON13350 DNA was digested with
NsiI and EcoRI. The resulting 111 base pair NsiI,
15 EcoRI fragment encodes amino acids 72-105 of (15-
125)hIL-3. The eluted restriction fragments were
concentrated and desalted using Centricon 30
concentrators. The restriction fragments were ligated,
and the ligation reaction mixture was used to transform
20 E. coli K-12 strain JM101. Transformant bacteria were
selected on ampicillin-containing plates. Plasmid DNA
was isolated and analyzed by restriction analysis.
Clones containing the correct insert lost a XmnI site
as compared with pMON13352. Positive clones were
25 identified by the loss of a 615 base pair XmnI
fragment. The DNA was sequenced to confirm the correct
insert. The resulting (15-125)hIL-3 variant has the
following amino acid substitutions: 73G, 76A, 79R,
82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and
30 123E. The codons encoding amino acids 72-125 of (15-
125)hIL-3 are those found in the hIL-3 cDNA sequence
except at those positions where amino acid
substitutions were made. The plasmid, pMON13360,
encodes the (15-125)hIL-3 variant with the following
35 amino acid sequence:

Peptide #12

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

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Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 5 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 10 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 15 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 20 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ. NO:76]

DNA sequence #23 [SEQ ID NO:119] encodes the
 foregoing pMON13360 polypeptide.
 25

EXAMPLE 22

Construction of pMON13361

Plasmid pMON13352 DNA was digested with restriction
 30 enzymes NsiI and EcoRI, resulting in a 4178 base pair
 NsiI, EcoRI fragment. The genetic elements derived from
 pMON13352 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage f1 origin of replication
 as the transcription terminator, pAraBAD promoter, g10L
 35 ribosome binding site, lamB secretion leader and the
 bases encoding amino acids 15-71 and 106-125 of (15-
 125)hIL-3. Plasmid pMON13355 DNA was digested with
 NsiI and EcoRI. The resulting 111 base pair NsiI,
 EcoRI fragment encodes amino acids 72-105 of (15-
 40 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Clones
 containing the correct insert contained an additional
 45 RsaI site which results in a 1200 base pairs RsaI

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fragment. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E.

5 The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13361, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #13**

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

15 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

20 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser

25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

30 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:77]

DNA sequence #24 [SEQ ID NO:120] codes for the foregoing pMON13361 polypeptide.

35

EXAMPLE 23

Construction of pMON13362

Plasmid pMON13354 DNA was digested with restriction
40 enzymes NsiI and EcoRI, resulting in a 4178 base pair NsiI,EcoRI fragment. The genetic elements derived from pMON13354 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication as the transcription terminator, pAraBAD promoter, g10L
45 ribosome binding site, lamB secretion leader and the

bases encoding amino acids 15-71 and 106-125 of (15-125)hIL-3. Plasmid pMON13355 DNA was digested with NsiI and EcoRI. The resulting 111 base pair NsiI, EcoRI fragment encodes amino acids 72-105 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Clones containing the correct insert contained an additional RsaI site which results in a 1200 base pairs RsaI fragment. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13362, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #14

Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:78]

DNA sequence #25 [SEQ ID NO:121] codes for the

foregoing pMON13362 polypeptide.

EXAMPLE 24

5 Construction of pMON13363

Plasmid pMON13344 DNA was digested with restriction enzymes NsiI and EcoRV, resulting in a 4218 base pair NsiI,EcoRV fragment. The genetic elements derived from pMON13344 are the beta-lactamase gene (AMP), pBR327
 10 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid pMON13348 DNA was digested with
 15 NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV fragment encodes amino acids 47-71 of (15-125)hIL-3. The restriction fragments were ligated with T4 ligase, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were
 20 selected on ampicillin-containing plates. Clones containing the correct insert contained an additional DdeI site which results in DdeI restriction fragments of 806 and 167 base pairs compared to 973 base pairs in pMON13344. The DNA was sequenced to confirm the
 25 correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence
 30 except at those positions where amino acid substitutions were made. The plasmid, pMON13363, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #15

35 Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

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Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 5 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 15 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:79]

20 **DNA sequence #20** [SEQ ID NO:116] codes for the
 foregoing pMON13363 polypeptide.

EXAMPLE 25

25 Construction of pMON13364
 Plasmid pMON13345 DNA was digested with restriction
 enzymes NsiI and EcoRV, resulting in a 4218 base pair
 NsiI,EcoRV fragment. The genetic elements derived from
 pMON13345 are the beta-lactamase gene (AMP), pBR327
 30 origin of replication, phage f1 origin of replication
 as the transcription terminator, pAraBAD promoter, g10L
 ribosome binding site, lamB secretion leader and the
 bases encoding amino acids 15-46 and 72-125 of (15-
 125)hIL-3. Plasmid pMON13349 DNA was digested with
 35 NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV
 fragment encodes amino acids 47-71 of (15-125)hIL-3.
 The restriction fragments were ligated, and the
 ligation reaction mixture was used to transform E. coli
 K-12 strain JM101. Transformant bacteria were selected
 40 on ampicillin-containing plates. Clones containing the
 correct insert contained an additional DdeI site which
 results in DdeI restriction fragments of 806 and 167
 base pairs compared to 973 base pairs in pMON13344.

The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13364, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

10 **Peptide #16**

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

15 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

20 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

25 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

30 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:80]

35 **DNA sequence #21** [SEQ ID NO:117] codes for the foregoing pMON13364 polypeptide.

EXAMPLE 26

Construction of pMON13365

40 Plasmid pMON13346 DNA was digested with restriction enzymes NsiI and EcoRV, resulting in a 4218 base pair NsiI,EcoRV fragment. The genetic elements derived from pMON13346 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication
45 as the transcription terminator, pAraBAD promoter, g10L

ribosome binding site, lamB secretion leader and the bases encoding amino acids 15-46 and 72-125 of (15-125)hIL-3. Plasmid pMON13347 DNA was digested with NsiI and EcoRV. The resulting 71 base pair NsiI, EcoRV fragment encodes amino acids 47-71 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Clones containing the correct insert contained an additional DdeI site which results in DdeI restriction fragments of 806 and 167 base pairs compared to 973 base pairs in pMON13344. The DNA was sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13365, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #17

Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:81]

DNA sequence #22 [SEQ ID NO:118] codes for the

foreging pMON13365 polypeptide.

EXAMPLE 27

5 Construction of pMON13298

Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair NsiI,HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327
 10 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter, g10L ribosome binding site, and the bases encoding amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13360 DNA was digested with NsiI and HindIII. The resulting 175
 15 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-
 20 containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q
 25 and 123E. The codons encoding amino acids 72-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13298, encodes the (15-125)hIL-3 variant with the following
 30 amino acid sequence:

Peptide #18

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu.

35 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

40

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Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:82]
 15 **DNA sequence #29** [SEQ ID NO:125] codes for the
 foregoing pMON13298 polypeptide.

EXAMPLE 28

20 Construction of pMON13299
 Plasmid pMON5978 DNA was digested with restriction
 enzymes NsiI and HindIII, resulting in a 3789 base pair
 NsiI,HindIII fragment. The genetic elements derived
 from pMON5978 are the beta-lactamase gene (AMP), pBR327
 25 origin of replication, phage f1 origin of replication
 as the transcription terminator, preCA promoter, gl0L
 ribosome binding site and the bases encoding amino
 acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA
 was digested with NsiI and HindIII, the resulting 175
 30 base pair NsiI, HindIII fragment encodes amino acids
 72-125 of (15-125)hIL-3. The restriction fragments
 were ligated, and the ligation reaction mixture was
 used to transform E. coli K-12 strain JM101.
 Transformant bacteria were selected on ampicillin-
 35 containing plates. Plasmid DNA was isolated, analyzed
 by restriction analysis, and sequenced to confirm the
 correct insert. The resulting (15-125)hIL-3 variant
 has the following amino acid substitutions: 73G, 76A,
 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q
 40 and 123E. The codons encoding amino acids 72-125 of
 (15-125)hIL-3 are those found in the hIL-3 cDNA

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sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13299, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

5 **Peptide #19**

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
 10 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly
 Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn
 15 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 25 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:83]

DNA sequence #30 [SEQ ID NO:126] codes for the foregoing pMON13299 polypeptide.

30

EXAMPLE 29

Construction of pMON13300

35 Plasmid pMON5978 DNA was digested with restriction enzymes NsiI and HindIII, resulting in a 3789 base pair NsiI,HindIII fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage fl origin of replication
 40 as the transcription terminator, preCA promoter, g10L ribosome binding site, and the bases encoding amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids
 45 72-125 of (15-125)hIL-3. The restriction fragments

were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101.

Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed
 5 by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 72-125
 10 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13300, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

15 **Peptide #20**

Met Ala Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu

20 Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly

Glu Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn

25 Leu Glu Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser

30 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser

Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:84]

DNA sequence #31 [SEQ ID NO:127] codes for the
 40 foregoing pMON13300 polypeptide.

EXAMPLE 30

Construction of pMON13301

45 Plasmid pMON5978 DNA was digested with restriction

enzymes NcoI and NsiI, resulting in a 3794 base pair NcoI,NsiI fragment. The genetic elements derived from pMON5978 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter, g10L ribosome binding site and the bases encoding amino acids 72-125 of (15-125)hIL-3. Plasmid pMON13363 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N and 69E. The codons encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13301, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #21

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

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Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:85]

DNA sequence #26 [SEQ ID NO:122] codes for the foregoing pMON13301 polypeptide.

5

EXAMPLE 31Construction of pMON13302

Plasmid pMON5978 DNA was digested with restriction
 10 enzymes NcoI and NsiI, resulting in a 3794 base pair
 NcoI,NsiI fragment. The genetic elements derived from
 pMON5978 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage fl origin of replication
 as the transcription terminator, precA promoter, gl0L
 15 ribosome binding site, and the bases encoding amino
 acids 72-125 of (15-125)hIL-3. Plasmid pMON13364 DNA
 was digested with NcoI and NsiI. The resulting 170
 base pair NcoI, NsiI fragment encodes amino acids 15-71
 of (15-125)hIL-3. The restriction fragments were
 20 ligated, and the ligation reaction mixture was used to
 transform E. coli K-12 strain JM101. Transformant
 bacteria were selected on ampicillin-containing plates.
 Plasmid DNA was isolated, analyzed by restriction
 analysis, and sequenced to confirm the correct insert.
 25 The resulting (15-125)hIL-3 variant has the following
 amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S,
 45M, 51R, 55T, 59L, 62V, 67H and 69E. The codons
 encoding amino acids 15-71 of (15-125)hIL-3 are those
 found in the hIL-3 cDNA sequence except at those
 30 positions where amino acid substitutions were made.
 The plasmid, pMON13302, encodes the (15-125)hIL-3
 variant with the following amino acid sequence:

Peptide #22

35 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

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Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 5 Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu
 10 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly
 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr
 15 Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:86]

DNA sequence #27 [SEQ ID NO:123] codes for the
 foregoing pMON13302 polypeptide.
 20

EXAMPLE 32

Construction of pMON13303

Plasmid pMON5978 DNA was digested with restriction
 25 enzymes NcoI and NsiI, resulting in a 3794 base pair
 NcoI, NsiI fragment. The genetic elements derived from
 pMON5978 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage fl origin of replication
 as the transcription terminator, preCA promoter, g10L
 30 ribosome binding site, and the bases encoding amino
 acids 72-125 of (15-125)hIL-3. Plasmid pMON13365 DNA
 was digested with NcoI and NsiI. The resulting 170
 base pair NcoI, NsiI fragment encodes amino acids 15-71
 of (15-125)hIL-3. The restriction fragments were
 35 ligated, and the ligation reaction mixture was used to
 transform E. coli K-12 strain JM101. Transformant
 bacteria were selected on ampicillin-containing plates.
 Plasmid DNA was isolated, analyzed by restriction
 analysis, and sequenced to confirm the correct insert.
 40 The resulting (15-125)hIL-3 variant has the following
 amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S,
 45M, 51R, 55L, 59L, 62V, 67N and 69E. The codons

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encoding amino acids 15-71 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made.

The plasmid, pMON13303, encodes the (15-125)hIL-3

5 variant with the following amino acid sequence:

Peptide #23

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

10 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

15 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu

20 Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly

25 Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr

Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:87]

30 **DNA sequence #28** [SEQ ID NO:124] codes for the foregoing pMON13303 polypeptide.

EXAMPLE 33

35 Construction of pMON13287

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327

40 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13363 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of

45 (15-125)hIL-3. Plasmid pMON13360 DNA was digested with

NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform

5 E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid

10 substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those

15 positions where amino acid substitutions were made. The plasmid, pMON13287, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #24

20 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala

25 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

30 Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

35 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

40 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:88]

DNA sequence #1 [SEQ ID NO:97] codes for the foregoing pMON13287 polypeptide.

EXAMPLE 34Construction of pMON13288

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and gl0L ribosome binding site. Plasmid pMON13364 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13360 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13288, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

30 **Peptide #25**

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

35 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

40 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser

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Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser
5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
10 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:89]

DNA sequence #4 [SEQ ID NO:100] codes for the
foregoing pMON13288 polypeptide.
15

EXAMPLE 35

Construction of pMON13289

Plasmid pMON2341 DNA was digested with restriction
20 enzymes NcoI and HindIII, resulting in a 3619 base pair
NcoI, HindIII fragment. The genetic elements derived
from pMON2341 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage fl origin of replication
as the transcription terminator, precA promoter and
25 g10L ribosome binding site. Plasmid pMON13365 DNA was
digested with NcoI and NsiI. The resulting 170 base
pair NcoI, NsiI fragment encodes amino acids 15-71 of
(15-125)hIL-3. Plasmid pMON13360 DNA was digested with
NsiI and HindIII. The resulting 175 base pair NsiI,
30 HindIII fragment encodes amino acids 72-125 of (15-
125)hIL-3. The restriction fragments were ligated, and
the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
selected on ampicillin-containing plates. Plasmid DNA
35 was isolated, analyzed by restriction analysis, and
sequenced to confirm the correct insert. The resulting
(15-125)hIL-3 variant has the following amino acid
substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R,
55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82Q, 87S, 93S,
40 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons

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encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made.

The plasmid, pMON13289, encodes the (15-125)hIL-3

5 variant with the following amino acid sequence:

Peptide #26

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

10 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

15 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser

Gly Ile Glu Ala Ile Leu Arg Asn Leu Gln Pro Cys Leu Pro Ser

20 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Ile Ile Lys Ala Gly

25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr

Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:90]

30 **DNA sequence #7** [SEQ ID NO:103] codes for the foregoing pMON13289 polypeptide.

35 EXAMPLE 36

Construction of pMON13290

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived

40 from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, preCA promoter and g10L ribosome binding site. Plasmid pMON13363 DNA was digested with NcoI and NsiI. The resulting 170 base

45 pair NcoI, NsiI fragment encodes amino acids 15-71 of

(15-125)hIL-3. Plasmid pMON13361 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R, 55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13290, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #27

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:91]

DNA sequence #2 [SEQ ID NO:98] codes for the foregoing pMON13290 polypeptide.

EXAMPLE 37Construction of pMON13292

Plasmid pMON2341 DNA was digested with restriction
 5 enzymes NcoI and HindIII, resulting in a 3619 base pair
 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 origin of replication, phage f1 origin of replication
 as the transcription terminator, precA promoter and
 10 gl0L ribosome binding site. Plasmid pMON13365 DNA was
 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 (15-125)hIL-3. Plasmid pMON13361 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 15 HindIII fragment encodes amino acids 72-125 of (15-
 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform
E. coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 20 was isolated, analyzed by restriction analysis, and
 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R,
 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S,
 25 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons
 encoding amino acids 15-125 of (15-125)hIL-3 are those
 found in the hIL-3 cDNA sequence except at those
 positions where amino acid substitutions were made.
 The plasmid, pMON13292, encodes the (15-125)hIL-3
 30 variant with the following amino acid sequence:

Peptide #28

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

35 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn

240

Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 5 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
 Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:92]

15 **DNA sequence #8** [SEQ ID NO:104] codes for the
 foregoing pMON13292 polypeptide.

EXAMPLE 38

20 Construction of pMON13294
 Plasmid pMON2341 DNA was digested with restriction
 enzymes NcoI and HindIII, resulting in a 3619 base pair
 NcoI,HindIII fragment. The genetic elements derived
 from pMON2341 are the beta-lactamase gene (AMP), pBR327
 25 origin of replication, phage fl origin of replication
 as the transcription terminator, preCA promoter and
 g10L ribosome binding site. Plasmid pMON13364 DNA was
 digested with NcoI and NsiI. The resulting 170 base
 pair NcoI, NsiI fragment encodes amino acids 15-71 of
 30 (15-125)hIL-3. Plasmid pMON13362 DNA was digested with
 NsiI and HindIII. The resulting 175 base pair NsiI,
 HindIII fragment encodes amino acids 72-125 of (15-
 125)hIL-3. The restriction fragments were ligated, and
 the ligation reaction mixture was used to transform E.
 35 coli K-12 strain JM101. Transformant bacteria were
 selected on ampicillin-containing plates. Plasmid DNA
 was isolated, analyzed by restriction analysis, and
 sequenced to confirm the correct insert. The resulting
 (15-125)hIL-3 variant has the following amino acid
 40 substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R,
 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S,

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98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made.

- 5 The plasmid, pMON13294, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #29

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 10 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn
 15 Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 25 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:93]

30

DNA sequence #6 [SEQ ID NO:102] codes for the foregoing pMON13294 polypeptide.

35

EXAMPLE 39

Construction of pMON13295

Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair
 40 NcoI, HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13365 DNA was
 45 digested with NcoI and NsiI. The resulting 170 base

pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13362 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29V, 32A, 37S, 42S, 45M, 51R, 55L, 59L, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13295, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #30

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Val Pro Pro Ala Pro Leu Leu Asp Ser Asn Asn Leu Asn Ser
 Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 Leu Leu Ala Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:94]

DNA sequence #9 [SEQ ID NO:105] codes for the foregoing pMON13295 polypeptide.

EXAMPLE 40Construction of pMON13312

- 5 Plasmid pMON2341 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3619 base pair NcoI,HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication
- 10 as the transcription terminator, precA promoter and g10L ribosome binding site. Plasmid pMON13364 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes amino acids 15-71 of (15-125)hIL-3. Plasmid pMON13361 DNA was digested with
- 15 NsiI and HindIII. The resulting 175 base pair NsiI, HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were
- 20 selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis, and sequenced to confirm the correct insert. The resulting (15-125)hIL-3 variant has the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R,
- 25 55T, 59L, 62V, 67H, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 120Q and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made.
- 30 The plasmid, pMON13312, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #31

Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu

- 35 Lys Arg Pro Pro Asn Pro Leu Leu Asp Pro Asn Asn Leu Asn Ser

Glu Asp Met Asp Ile Leu Met Glu Arg Asn Leu Arg Thr Pro Asn

Leu Leu Ala Phe Val Arg Ala Val Lys His Leu Glu Asn Ala Ser
5 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
10 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Thr
Leu Glu Gln Ala Gln Glu Gln Gln [SEQ ID NO:95]
15

DNA sequence #5 [SEQ ID NO:101] codes for the
foregoing pMON13312 polypeptide.

20

EXAMPLE 41

Construction of pMON13313

Plasmid pMON2341 DNA was digested with restriction
enzymes NcoI and HindIII, resulting in a 3619 base pair
25 NcoI, HindIII fragment. The genetic elements derived
from pMON2341 are the beta-lactamase gene (AMP), pBR327
origin of replication, phage f1 origin of replication
as the transcription terminator, precA promoter and
g10L ribosome binding site. Plasmid pMON13363 DNA was
30 digested with NcoI and NsiI. The resulting 170 base
pair NcoI, NsiI fragment encodes amino acids 15-71 of
(15-125)hIL-3. Plasmid pMON13362 DNA was digested with
NsiI and HindIII. The resulting 175 base pair NsiI,
HindIII fragment encodes amino acids 72-125 of (15-
35 125)hIL-3. The restriction fragments were ligated, and
the ligation reaction mixture was used to transform E.
coli K-12 strain JM101. Transformant bacteria were
selected on ampicillin-containing plates. Plasmid DNA
was isolated, analyzed by restriction analysis, and
40 sequenced to confirm the correct insert. The resulting
(15-125)hIL-3 variant has the following amino acid
substitutions: 18I, 25H, 29R, 32A, 37P, 42A, 45V, 51R,

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55L, 60S, 62V, 67N, 69E, 73G, 76A, 79R, 82V, 87S, 93S, 98T, 101A, 105Q, 109E, 116V, 117S, 120H and 123E. The codons encoding amino acids 15-125 of (15-125)hIL-3 are those found in the hIL-3 cDNA sequence except at those

5 positions where amino acid substitutions were made.

The plasmid, pMON13313, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

Peptide #32

10 Met Ala Asn Cys Ser Ile Met Ile Asp Glu Ile Ile His His Leu
 Lys Arg Pro Pro Ala Pro Leu Leu Asp Pro Asn Asn Leu Asn Ala
 15 Glu Asp Val Asp Ile Leu Met Glu Arg Asn Leu Arg Leu Pro Asn
 Leu Glu Ser Phe Val Arg Ala Val Lys Asn Leu Glu Asn Ala Ser
 20 Gly Ile Glu Ala Ile Leu Arg Asn Leu Val Pro Cys Leu Pro Ser
 25 Ala Thr Ala Ala Pro Ser Arg His Pro Ile Thr Ile Lys Ala Gly
 Asp Trp Gln Glu Phe Arg Glu Lys Leu Thr Phe Tyr Leu Val Ser
 30 Leu Glu His Ala Gln Glu Gln Gln [SEQ ID NO:96]

DNA sequence #3 [SEQ ID NO:99] codes for the foregoing pMON13313 polypeptide.

35 EXAMPLE 42

Construction of pMON5987

Plasmid pMON6458 DNA was digested with restriction enzymes NcoI and HindIII, resulting in a 3940 base pair
 40 NcoI,HindIII fragment. The genetic elements derived from pMON6458 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, pAraBAD promoter, g10L ribosome binding site and lamB secretion leader.
 45 Plasmid pMON5978 DNA was digested with NcoI and NsiI. The resulting 170 base pair NcoI, NsiI fragment encodes

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amino acids 15-71 of (15-125)hIL-3. Plasmid pMON5976 DNA was digested with NsiI and HindIII. The resulting 175 base pair NsiI,HindIII fragment encodes amino acids 72-125 of (15-125)hIL-3. The restriction fragments
5 were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and screened for the restriction sites EcoRV and NheI and
10 DNA sequenced to confirm the correct insert.

EXAMPLE 43

Construction of pMON5988

15 The plasmid DNA of pMON5987 was digested with NheI and EcoRI, resulting in a 3903 base pair NheI, EcoRI fragment. The 3903 base pair NheI, EcoRI fragment was ligated to 1.0 picomoles of the following annealed oligonucleotides:

20

5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAA-
3'-GGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTATAGTT-

GGACGGTGACTGGAATG-3' [SEQ ID NO:131]
25 CCTGCCACTGACCTTACAATT-5' [SEQ ID NO:132]

The ligation reaction mixture was used to transform E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates.
30 Plasmid DNA was isolated and sequenced to confirm positive clones. This plasmid was constructed to change alanine 101 to aspartic acid in the hIL-3 gene (15-125). This plasmid was designated pMON5988.

35

EXAMPLE 44

Construction of pMON5853 (Fig 6) which encodes [Met-(15-133)hIL-3(Arg¹²⁹)]

Plasmid DNA of pMON5847 (Example 2) was treated with NcoI. The restriction enzyme was inactivated by
5 heat treatment (65°C for 10 minutes). The DNA was then treated with large fragment of DNA polymerase I (Klenow) in the presence of all four nucleotide precursors. This produces DNA termini with non-overlapping ends. After 5 minutes at 37°C, the
10 polymerase was inactivated by heat treatment at 65°C for 10 minutes. The DNA was then treated with HpaI, an enzyme which produces non-overlapping termini. The DNA was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101
15 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis. A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction
20 of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:133]

M N C S N [SEQ ID NO:134]

25

The lower line contains the one letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and
30 asparagine.

When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

35

The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg¹²⁹) which has the following amino acid sequence:

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Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 5 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg
 10 Leu Ala Ile Phe [SEQ ID NO:135]

EXAMPLE 45

Construction of pMON5873 which encodes [Met-(1-133)hIL-
 15 3]

The gene obtained from British Biotechnology, Ltd. specified arginine at codon position 129. The amino acid specified in the native hIL-3 cDNA is serine. To produce a protein with the native sequence at this
 20 position, the portion of the coding sequence between the EcoRI site at codons 106 and 107 and the NheI site at codons 129 and 130 was replaced. Plasmid DNA of pMON5854 (Example 3) and pMON5853 (Example 44) were treated with EcoRI and NheI. The larger fragments of
 25 each were gel purified. These were ligated to a pair of an annealed oligonucleotides with the following sequences:

5'-AATTCGTCGTAAGCTGACCTTCTATCTGAAAACC-
 30 3'-GGCAGCATTTGACTGGAAGATAGACTTTTGG-

TTGGAGAACGCGCAGGCTCAACAGACCACTCTGTCTG-3' [SEQ ID NO:
 136]
 AACCTCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATC-5' [SEQ ID
 35 NO:137]

The ligation reaction mixtures were used to transform competent JM101 cells to ampicillin resistance. Colonies were picked into broth and grown. Plasmid DNA was isolated and screened for the presence of a new StyI recognition site present in the synthetic DNA and not in pMON5854 and pMON5853. The nucleotide sequence of the gene in the region between EcoRI and NheI was determined and found to be that of the synthetic oligonucleotides. The new plasmids were designated pMON5873 encoding [Met-(1-133)hIL-3] and pMON5872 encoding [Met-(15-133)hIL-3].

The plasmid, pMON5873, encodes Met-(1-133)hIL-3 which has the following amino acid sequence:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser
 15 Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 20 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser
 Leu Ala Ile Phe [SEQ ID NO:128]

25

EXAMPLE 46

Construction of pMON6458

Plasmid pMON6525 was digested with restriction enzymes HindIII and SalI and the resulting 3172 base pair
 30 fragment was isolated from a 1% agarose gel by interception onto DEAE membrane. The genetic elements derived from pMON6525 are the beta-lactamase gene (AMP), pBR327 origin of replication, and phage f1
 35 origin of replication as the transcription terminator. (The genetic elements derived from plasmid pMON6525 are

identical to those in plasmid pMON2341 which could also be used to construct pMON6458.) Plasmid pMON6457 was digested with restriction enzymes HindIII and SalI and the resulting 1117 base pair fragment was isolated by
5 PAGE and crush and soak elution. The genetic elements derived from pMON6457 are the pAraBAD promoter, g10L ribosome binding site, lamB secretion leader and the (15-125) hIL-3 gene. The restriction fragments were ligated and the ligation reaction mixture was used to
10 transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in
15 double digest. Clones containing the hIL-3 gene (encoding amino acids 15-125) contained a 345 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6458. This plasmid was constructed to eliminate an EcoRI restriction site outside the hIL-3
20 gene coding region in plasmid pMON6457.

EXAMPLE 47

Construction of pMON5976 which encodes [Met-(15-125)hIL-3(Ala101)]
25

The plasmid DNA of pMON5941 isolated from the dam-
E. coli strain GM48 was cleaved with ClaI and NsiI and ligated to 1 picomole of an annealed assembly of six
oligonucleotides encoding amino acids 20-70 of hIL-3
30 (FIG. 2). This synthetic fragment encodes three unique restriction sites, EcoRV, XhoI and PstI. The sequence of these oligonucleotides is shown in Figure 2.

The resulting ligation mix was used to transform competent E. coli JM101 cells to ampicillin resistant
35 colonies. Plasmid DNA was isolated and the inserted fragment was determined to have both an EcoRV and NheI

site. The nucleotide sequence of the region between
 ClaI and NsiI was determined and found to be that of
 the synthetic oligonucleotides. At codons 86-87 of a
 nucleotide sequence coding for (15-125)hIL-3, an NheI
 5 site was introduced. The plasmid with this alteration
 was designated pMON5941. This plasmid encodes Met-(15-
 125)hIL-3 which is altered at position 101 by
 replacement of aspartate by alanine.

10 Plasmid pMON5976 encodes Met-(15-125)hIL-3(Ala¹⁰¹)
 which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 15 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Ala Gly Asp
 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 20 Thr Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:138]

EXAMPLE 48

Construction of pMON5917 which encodes [Met-(15-88)hIL-
 25 3]

The plasmid DNA of pMON5853 was cleaved with NsiI
 and HindIII and ligated to an annealed pair of
 oligonucleotides encoding (70-88)hIL-3 with a new NheI
 endonuclease restriction site at codons 86-87. The
 30 sequence of these oligonucleotides is shown in
 Example 18.

The ligation mixture was used to transform
 competent *E. coli* JM101 cells, and ampicillin resistant
 colonies were picked. Plasmid DNA isolated from
 35 individual colonies was screened for the presence of
 the new NheI restriction site. The nucleotide sequence

of the substituted portion was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5917 encoding Met-(15-88)hIL-3 containing a new NheI site at codons 86-87.

5 Plasmid pMON5917 encodes Met-(15-88)hIL-3 which has the following amino acid sequence:

Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
10 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala [SEQ ID NO:
139]

15

EXAMPLE 49

Construction of pmon5941 which encodes [Met-(15-125)hIL-3 Ala¹⁰¹]

The plasmid DNA of pMON5917 was cleaved with NheI
20 and HindIII and ligated to two annealed pairs of oligonucleotides which encode amino acids 86-106 and 107-125 of hIL-3. The sequences of these oligonucleotides is shown below.

NheI to EcoRI

25 5'-CTAGCCACGGCCGCACCCACGCGACATCCAATCCATATCAAGGCTG-
3'-GGTGCCGGCGTGCGTGTAGGTTAGGTATAGTTCCGAC-

GTGACTGGAATG-3' [SEQ ID NO:140]

CACTGACCTTACTTAA-5' [SEQ ID NO:141]

30

EcoRI to HindIII

5'-AATTCCGTCGTAAACTGACCTTCTATCTGAAAACCTTGGAGAACGCGCA-
3'-GGCAGCATTGACTGGAAGATAGACTTTTGAACCTCTTGCGCGT-

35 GGCTCAACAGTAATA-3' [SEQ ID NO:142]

CCGAGTTGTCATTATTCGA-5' [SEQ ID NO:143]

The ligation mixture was used to transform competent E. coli JM101 cells to ampicillin resistant colonies. Plasmid DNA was isolated from these cells and the size of the inserted fragment was determined to be larger by restriction analysis with NcoI and HindIII. The Asp to Ala 101 change is encoded on the NheI to EcoRI fragment. The nucleotide sequence of the portion of the coding region between the NheI and HindIII sites was determined and found to be that of the synthetic oligonucleotides. The new plasmid was designated pMON5941.

The plasmid, pMON5941, encodes Met-(15-125)hIL-3(Ala101) and contains a new NheI restriction site.

EXAMPLE 50

Construction of pMON6455

Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, pAraBAD promoter, gl0L ribosome binding site, lamB secretion leader and phage fl origin of replication as the transcription terminator. The following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, gl0L ribosome binding site and phage fl origin of replication as the transcription terminator, derived from plasmid pMON5905 are identical to these in plasmid pMON5594 which could also be used to construct pMON6455. The AraBAD promoter is identical to that described in pMON6235. The lamB signal peptide sequence used in pMON6455 is that shown in Figure 8 fused to hIL-3 (15-125) at the NcoI site. Plasmid pMON5887 was digested with restriction enzymes HindIII and NcoI, resulting in a

254

384 base pair NcoI, HindIII fragment. The restriction fragments were ligated, and the ligation reaction mixture was used to transform into E. coli K-12 strain JM101. Transformant bacteria were selected on
5 ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Positive clones
10 containing the hIL-3 gene (encoding amino acids 1-125) contained a 384 base pair NcoI, HindIII restriction fragment. This construct was designated pMON6455.

EXAMPLE 51

Construction of pMON6456

15 Plasmid pMON5905 was digested with restriction enzymes HindIII and NcoI resulting in a 3936 base pair fragment. The genetic elements derived from pMON5905 are the beta-lactamase gene (AMP), pBR327 origin of replication, phage f1 origin of replication as the
20 transcription terminator, pAraBAD promoter, gl0L ribosome binding site and the lamB secretion leader. Plasmid pMON5871 was digested with restriction enzymes HindIII and NcoI, resulting in a 330 base pair NcoI, HindIII fragment. The genetic element derived from
25 pMON5871 encompassed the bases encoding the (1-107) hIL-3 gene. The restriction fragments were ligated, and the ligation reaction mixture was used to transform E. coli K-12 strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA
30 was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes NcoI and HindIII in double digest. Clones containing the hIL-3 gene (encoding amino acids 1-107) contained a 330 base pair NcoI, HindIII
35 restriction fragment. This construct was designated pMON6456.

EXAMPLE 525 Construction of pMON6457

Plasmid pMON6455 DNA grown in E. coli strain GM48 (dam-) was digested with restriction enzymes NcoI and ClaI, resulting in a 4263 base pair NcoI, ClaI fragment. The restriction fragment was ligated to 1.0 picomoles of annealed oligonucleotides with the following sequence coding for Met Ala 14-20 hIL-3:

5'-CATGGCTAACTGCTCTAACATGAT-3' [SEQ ID NO:151]

3'-CGATTGACGAGATTGTACTAGC-5' [SEQ ID NO:152]

15

The resulting DNA was transformed into E. coli K-12 strain JM101 and transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated and the size of the inserted fragment was determined by restriction analysis employing restriction enzymes XbaI and EcoRI in double digest. Positive clones containing the hIL-3 gene (encoding aa 15-125 of hIL-3) contained a 433 base pair XbaI, EcoRI restriction fragment. This construct was designated pMON6457. This plasmid was constructed to delete the first 14 amino acids of hIL-3. The coding sequence of the resulting gene begins as follows:

5' ATG GCT AAC TGC... 3' [SEQ ID NO:153]
30 Met Ala Asn Cys... [SEQ ID NO:154]

15

The first two amino acids (Methionine, Alanine) create an NcoI restriction site and a signal peptidase cleavage site between the lamB signal peptide and (15-125) hIL-3. Plasmid pMON6457 encodes (15-125) hIL-3

35

which has the amino acid sequence designated SEQ ID NO:65.

5

EXAMPLE 53Construction of pMON6235

One of the DNA fragments used to create this plasmid was generated by site-directed mutagenesis employing PCR techniques described previously using the following
10 oligonucleotides, Oligo #51 [SEQ ID NO:155] and Oligo #52 [SEQ ID NO:156], were used as primers in this procedure. The template for the PCR reaction was E. coli strain W3110 chromosomal DNA, prepared as described in Maniatis (1982). The oligonucleotide
15 primers were designed to amplify the AraBAD promoter (Greenfield et al., 1978). The resulting DNA product was digested with the restriction enzymes SacII and BglII. The reaction mixture was purified as described previously. Plasmid, pMON5594, DNA was digested with
20 SacII and BglII, resulting in a 4416 base pair SacII,BglII restriction fragment which contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, G10L ribosome binding site, phage f1 origin of replication as the
25 transcription terminator and the chloramphenicol acetyl transferase (cat) gene. The 4416 base pair SacII,BglII restriction fragment from pMON5594 was ligated to the PCR-generated SacII, BglII DNA fragment. The ligation mixture was used to transform E. coli K-112 strain
30 JM101. Positive clones contained a 323 base pair SacII,BglII fragment and were DNA sequenced to confirm that the SacII,BglII fragment was the AraBAD promoter. This construct was designated pMON6235.

35

EXAMPLE 54

Construction of pMON5647

Plasmid pMON5585 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3273 base pair NcoI,HindIII fragment. The genetic elements derived from pMON5585 are the pBR327 origin of replication, precA promoter, gl0L ribosome binding protein, bovine somatotropin gene (bST), beta-lactamase gene (AMP) and T7 transcription terminator. Plasmid pMON3267 [prepared as disclosed in EP 0241446 incorporated herein by reference in its entirety] DNA was digested with NcoI and HindIII enzymes resulting in a 580 base pair NcoI,HindIII fragment which contains the porcine somatotropin (pST) gene. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. Transformant bacteria were selected on ampicillin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert.

EXAMPLE 55Construction of pMON710

Plasmid pMON709 consists of a 1614 base pair AvaI,EcoRI fragment of transposon TN7, containing the streptomycin adenylyltransferase gene (Fling et al., 1985) and a pUC9 linker (XmaI,HindIII) cloned between the HindIII and EcoRI sites of pUC19. The streptomycin adenylyltransferase gene confers resistance to streptomycin and spectinomycin. Plasmid pMON709 was mutagenized by oligonucleotide site-directed mutagenesis (methods described in Zoller and Smith, 1982) to introduce an EcoRV site at the 3' end of the streptomycin adenylyltransferase gene. The oligonucleotide, **Oligo # 53** [SEQ ID NO:157], was used

in this procedure to introduce the EcoRV site. The resulting plasmid was designated pMON710.

5

EXAMPLE 56Construction of pMON5723

Plasmid pMON5647 DNA was digested with restriction enzymes DraI and SspI resulting in a 2916 base pair DraI, SspI fragment. The genetic elements derived from pMON5647 are the pBR327 origin of replication, preCA promoter, gl0L ribosome binding protein, porcine somatotropin gene (pST) and T7 transcription terminator (Dunn and Strudier, 1983). Plasmid pMON710 DNA was digested with restriction enzymes HincII and EcoRV resulting in 940 base pair HincII, EcoRV fragment containing the streptomycin adenyltransferase gene which confers resistance to streptomycin and spectinomycin. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. The DraI, SspI, HincII and EcoRV restriction sites are lost as a result of the cloning. Transformant bacteria were selected on spectinomycin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert.

25

EXAMPLE 57Construction of pMON13361

Plasmid pMON13288 was mutagenized by oligonucleotide site-directed mutagenesis (method described in Kunkel, 1985) to eliminate a NsiI site in the (15-125) hIL-3 variant coding region. Codon 70 of (15-125) hIL-3, encoding asparagine, was converted from AAT to AAC destroying the NsiI recognition site. The oligonucleotide, **Oligo # 54** [SEQ ID NO:158], was used

35

in this procedure to eliminate the NsiI site.
Transformant bacteria were selected on ampicillin-
containing plates. Plasmid DNA was isolated, analyzed
by restriction analysis to confirm the loss of the NsiI
5 site and sequenced to confirm the sequence of the (15-
125) hIL-3 variant gene. The plasmid, pMON13361,
encodes the (15-125) hIL-3 variant with the amino acid
sequence of PEPTIDE #25 [SEQ ID NO:89].
DNA sequence # 32 [SEQ ID NO:160] codes for the
10 foregoing pMON13361 polypeptide.

EXAMPLE 58

Construction of pMON14058

15 Plasmid pMON13361 was mutagenized by oligonucleotide
site-directed mutagenesis (method described by Taylor
et al., 1985 using a kit from Amersham, Arlington
Heights, Ill.) to eliminate a EcoRV site in the (15-
125) hIL-3 variant coding region. Codon 46 and 47 of
20 (15-125) hIL-3, encoding asparagine and isoleucine,
were converted from GAT to GAC and ATC to ATT
respectively, destroying the EcoRV recognition site.
The oligonucleotide, **Oligo # 55** [SEQ ID NO:159], was
used in this procedure to eliminate the EcoRV site.
25 Transformant bacteria were selected on ampicillin-
containing plates. Plasmid DNA was isolated, analyzed
by restriction analysis to confirm the loss of the
EcoRV site and sequenced to confirm the sequence of the
(15-125) hIL-3 variant gene. The plasmid, pMON14058,
30 encodes the (15-125) hIL-3 variant with the amino acid
sequence of **PEPTIDE #25** [SEQ ID NO:89].
DNA sequence # 33 [SEQ ID NO:161] codes for the
foregoing pMON14058 polypeptide.

EXAMPLE 59

Construction of pMON13438

Plasmid pMON5723 DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3278 NcoI,HindIII fragment. The genetic elements derived from pMON5723 are the pBR327 origin of replication, precA promoter, g10L ribosome binding protein, T7 transcription terminator and streptomycin adenylyltransferase gene. Plasmid pMON14058 DNA was digested with NcoI and HindIII resulting in a 345 base pair NcoI,HindIII fragment which contains the (15-125) hIL-3 gene with the following amino acid substitutions: 18I, 25H, 29R, 32N, 37P, 42S, 45M, 51R, 55T, 59L, 62V, 67H, 69E,73G, 76A, 79R, 83Q, 87S, 93S, 98I, 101A, 105Q, 109E, 116V, 120Q and 123E. The restriction fragments were ligated and the ligation reaction mixture was used to transform E. coli strain JM101. Transformant bacteria were selected on spectinomycin-containing plates. Plasmid DNA was isolated, analyzed by restriction analysis and sequenced to confirm the correct insert. The plasmid, pMON13438, encodes the (15-125) hIL-3 variant with the amino acid sequence of **PEPTIDE #25** [SEQ ID NO:89]. **DNA sequence # 33** [SEQ ID NO:161] codes for the foregoing pMON13438 polypeptide.

25

EXAMPLE 60Construction of pMON13285

30

Plasmid pMON13252 DNA was digested with restriction enzymes NcoI and EcoRV and the resulting 3669 base pair NcoI,EcoRV fragment contains the following genetic elements; streptomycin adenylyltransferase gene, pBR327 origin of replication, phage f1 origin of replication as the transcription terminator, recA promoter, g10L ribosome binding site and the bases encoding amino

35

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acids 47-125 of (15-125) hIL-3 with the following amino acid substitution, 50D. The 3669 base pair NcoI,EcoRV restriction fragment from pMON13252 was ligated to the following annealed complementary oligonucleotides.

5

Oligo #165 [SEQ ID NO:162]

Oligo #166 [SEQ ID NO:163]

Oligo #167 [SEQ ID NO:164]

10 Oligo #168 [SEQ ID NO:165]

Oligo #169 [SEQ ID NO:166]

Oligo #170 [SEQ ID NO:167]

15 When assembled, the oligonucleotides create NcoI and EcoRV restriction ends and the DNA sequence that encodes amino acids 15-46 of (15-125) hIL-3 with the following amino acid substitutions; 42D, 45M and 46S. The codons encoding amino acids 15-46 of (15-125) hIL-3
20 are those found in the hIL-3 cDNA sequence except at those positions where amino acid substitutions were made. The plasmid, pMON13285, encodes the (15-125) hIL-3 variant with the following amino acid sequence:

25 Peptide #A3 [SEQ ID NO:258]

DNA sequence #A3 pMON13285 42D, 45M 46S, 50D

30 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT
CTATCCTGAT GGACAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
35 CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCAATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
40 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCGTCG TAAACTGACC

TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:398]

5

EXAMPLE 61Construction of pMON13286

Plasmid pMON5978 DNA was digested with restriction
10 enzymes NcoI and EcoRV and the resulting 3865 base pair
NcoI,EcoRV fragment contains the following genetic
elements; beta-lactamase gene (AMP), pBR327 origin of
replication, phage f1 origin of replication as the
transcription terminator, precA promoter, gl0L ribosome
15 binding site and the bases encoding amino acids 47-125
of (15-125) hIL-3. The 3865 base pair NcoI,EcoRV
restriction fragment from pMON5978 was ligated to the
following annealed complementary oligonucleotides.

20 Oligo #165 [SEQ ID NO:162]
Oligo #166 [SEQ ID NO:163]
Oligo #167 [SEQ ID NO:164]
Oligo #168 [SEQ ID NO:165]

25 Oligo #169 [SEQ ID NO:166]
Oligo #170 [SEQ ID NO:167]

When assembled, the oligonucleotides create NcoI and
EcoRV restriction ends and the DNA sequence that
30 encodes amino acids 15-46 of (15-125) hIL-3 with the
following amino acid substitutions; 42D, 45M and 46S.
The codons encoding amino acids 15-46 of (15-125) hIL-3
are those found in the hIL-3 cDNA sequence except at
those positions where amino acid substitutions were
35 made. The plasmid, pMON13286, encodes the (15-125)
hIL-3 variant with the following amino acid sequence:

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Peptide #A4 [SEQ ID NO:259]

DNA sequence #A4 pMON13286 42D, 45M, 46S

5 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGAC GAAGACATGT
10 CTATCCTGAT GGAAAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
CGTGCTGTCA AGTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
15 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
TTCTATCTGA AAACCTTGGA GAACGCGCAG GCTCAACAG
[SEQ ID NO:399]

20

EXAMPLE 62Construction of pMON13325

25 The 3704 base pair EcoRI, HindIII DNA fragment from
plasmid pMON13286 is ligated to the 64 base pair EcoRI,
HindIII DNA fragment from plasmid pMON13215. The
following genetic elements are derived from pMON13286;
beta-lactamase gene (AMP), pBR327 origin of
30 replication, phage F1 origin of replication as the
transcription terminator, preC promoter, g10L ribosome
binding site and the bases encoding amino acids 15-105
of the (15-125) hIL-3 gene with the following changes,
42D, 45M, and 46S. The bases encoding amino acids
35 106-125 of the (15-125) gene with the following change,
116W, are derived from pMON13215. The resulting
plasmid, pMON13325, encodes the (15-125) hIL-3 variant
with the following amino acid sequence:

40 Peptide # A5 [SEQ ID NO:261]

EXAMPLE 63Construction of pMON13326

5 The 3683 base pair NcoI, EcoRI DNA fragment from
plasmid pMON13215 is ligated to the 281 base pair NcoI,
EcoRI DNA fragment from plasmid pMON13285. The
following genetic elements are derived from pMON13215;
10 beta-lactamase gene (AMP), pBR327 origin of
replication, phage F1 origin of replication as the
transcription terminator, precA promoter, g10L ribosome
binding site and the bases encoding amino acids 106-125
of the (15-125) hIL-3 gene with the following change,
15 116W. The bases encoding amino acids 15-105 of the
(15-125) gene with the following change, 42D, 45M, 46S
and 50D derived from pMON13285. The resulting plasmid,
pMON13326, encodes the (15-125) hIL-3 variant with the
following amino acid sequence:

20

Peptide # A6 [SEQ ID NO:262]

EXAMPLE 6425 Construction of pMON13332

Plasmid pMON13326 DNA is digested with restriction
enzymes NsiI and EcoRI and the resulting 3853 base pair
NsiI,EcoRI fragment contains the following genetic
30 elements; beta-lactamase gene (AMP), pBR327 origin of
replication, phage f1 origin of replication as the
transcription terminator, recA promoter, g10L ribosome
binding site and the bases encoding amino acids 15-71
and 106-125 of (15-125) hIL-3 gene with the following
35 changes 42D, 45M, 46S, 50D and 116W. The 3853 base
pair NsiI,EcoRI restriction fragment from pMON13326 is

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ligated to the following annealed complementary
oligonucleotides.

Oligo #15(A) [SEQ ID NO:168]

5

Oligo #16(A) [SEQ ID NO:169]

In the resulting plasmid the 111 bases between the NsiI
and EcoRI restriction sites in the (15-125) hIL-3 gene
10 are replaced with 24 bases from the above mentioned
oligonucleotides. This linker also creates a NdeI
recognition sequence.

EXAMPLE 65

15

Construction of pMON13330

The 3846 base pair PstI, EcoRI DNA fragment from
plasmid pMON13332 is ligated to the 118 base pair
20 PstI, EcoRI DNA fragment from plasmid pMON13305. The
following genetic elements are derived from pMON13332;
beta-lactamase gene (AMP), pBR327 origin of
replication, phage f1 origin of replication as the
transcription terminator, recA promoter, g10L ribosome
25 binding site and the bases encoding amino acids 15-69
and 106-125 of the (15-125) hIL-3 gene with the
following change, 42D, 45M, 46S, 50D and 116W. The
bases encoding amino acids 70-105 of the (15-125) gene
with the following change, 95R, 98I and 100R are
30 derived from pMON13305. The resulting plasmid,
pMON13330, encodes the (15-125) hIL-3 variant with the
following amino acid sequence:

Peptide # A7 [SEQ ID NO:263]

35

EXAMPLE 66

Construction of pMON13329

The 3846 base pair PstI, EcoRI DNA fragment from
5 plasmid pMON13332 is ligated to the 118 base pair
PstI, EcoRI DNA fragment from plasmid pMON13304. The
following genetic elements are derived from pMON13332;
beta-lactamase gene (AMP), pBR327 origin of
replication, phage fl origin of replication as the
10 transcription terminator, recA promoter, g10L ribosome
binding site and the bases encoding amino acids 15-69
and 106-125 of the (15-125) hIL-3 gene with the
following change, 42D, 45M, 46S, and 116W. The bases
encoding amino acids 70-105 of the (15-125) gene with
15 the following change, 98I and 100R are derived from
pMON13304. The resulting plasmid, pMON13329, encodes
the (15-125) hIL-3 variant with the following amino
acid sequence:

20 Peptide # A8 [SEQ ID NO:406]

EXAMPLE 67

Construction of pMON5853 (Fig 6) which encodes [Met-
25 (15-133)hIL-3(Arg129)]

Plasmid DNA of pMON5847 (Example 2) was treated
with NcoI. The restriction enzyme was inactivated by
heat treatment (65°C for 10 minutes). The DNA was then
30 treated with large fragment of DNA polymerase I
(Klenow) in the presence of all four nucleotide
precursors. This produces DNA termini with non-
overlapping ends. After 5 minutes at 37°C, the
polymerase was inactivated by heat treatment at 65°C
35 for 10 minutes. The DNA was then treated with HpaI, an
enzyme which produces non-overlapping termini. The DNA

was ethanol precipitated and ligated. The ligation reaction mixture was used to transform competent JM101 cells to ampicillin resistance. Colonies were picked and plasmid DNA was analyzed by restriction analysis.

- 5 A plasmid designated pMON5853 was identified as one containing a deletion of the amino terminal 14 codons of the hIL-3 gene. The DNA sequence for the junction of the ribosome binding site to the (15-133) hIL-3 gene was determined to be the following:

10

5'-AAGGAGATATATCCATGAACTGCTCTAAC-3' [SEQ ID NO:400]

M N C S N [SEQ ID NO:401]

- 15 The lower line contains the one-letter code for the amino acids specified by the coding sequence of the amino terminus of the 15-133 hIL-3 gene. These are methionine, asparagine, cysteine, serine and asparagine.

- 20 When cultures of JM101 cells harboring this plasmid were induced with nalidixic acid, it was found that hIL-3 (15-133) accumulated at levels higher than hIL-3 (pMON5847).

- 25 The plasmid, pMON5853, encodes Met-(15-133) hIL-3 (Arg129) which has the following amino acid sequence:

- Met Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr
 His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn
 30 Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn
 Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
 Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
 Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala
 Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp
 35 Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys
 Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Arg

Leu Ala Ile Phe [SEQ ID NO:402]

EXAMPLE 68

5 Construction of pMON13252

Plasmid, pMON2341, DNA was digested with restriction enzymes NcoI and HindIII resulting in a 3619 base pair NcoI/HindIII fragment. The genetic elements derived from pMON2341 are the beta-lactamase gene (AMP), pBR327 origin of replication F1 phage origin of replication as the transcription terminator, precA, g10L ribosome binding site. The plasmid encoding the hIL-3 (15-125) Asp⁽⁵⁰⁾ variant, was digested with NcoI and HindIII resulting in a 345 base pair NcoI/HindIII fragment. This 345 Base pair NcoI/HindIII fragment was ligated with the 3619 base pair fragment from pMON2341 and the ligation reaction mixture was used to transform E.coli K-12 strain JM101. Plasmid DNA was isolated and screened by restriction analysis using NcoI and HindIII. Positive clones contained a 345 base pair NcoI/HindIII fragment. This construct was designated pMON13252. The plasmid, pMON13252, encodes the (15-125)hIL-3 variant with the following amino acid sequence:

PEPTIDE A10; (15-125)HIL-3 Asp⁽⁵⁰⁾ pMON13252

| | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 30 | | Asn | Cys | Ser | Asn | Met | Ile | Asp | Glu | Ile | Ile | Thr | His | Leu | |
| | | 15 | | | | | 20 | | | | | 25 | | | |
| | Lys | Gln | Pro | Pro | Leu | Pro | Leu | Leu | Asp | Phe | Asn | Asn | Leu | Asn | Gly |
| | | 30 | | | | | 35 | | | | | 40 | | | |
| | Glu | Asp | Gln | Asp | Ile | Leu | Met | Asp | Asn | Asn | Leu | Arg | Arg | Pro | Asn |
| 35 | | 45 | | | | | 50 | | | | | 55 | | | |
| | Leu | Glu | Ala | Phe | Asn | Arg | Ala | Val | Lys | Ser | Leu | Gln | Asn | Ala | Ser |

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| | | | |
|---|---|-----|-----|
| | 60 | 65 | 70 |
| | Ala Ile Glu Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu | | |
| | 75 | 80 | 85 |
| | Ala Thr Ala Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly | | |
| 5 | 90 | 95 | 100 |
| | Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr | | |
| | 105 | 110 | 115 |
| | Leu Glu Asn Ala Gln Ala Gln Gln [SEQ ID NO:407] | | |
| | 120 | 125 | |

10 DNA sequence #A10 pMON13252 50D

15 ATGGCTAACT GCTCTAACAT GATCGATGAA ATCATCACCC ACCTGAAGCA
 GCCACCGCTG CCGCTGCTGG ACTTCAACAA CCTCAATGGT GAAGACCAAG
 ATATCCTGAT GGAACAATAAC CTTCGTCGTC CAAACCTCGA GGCATTCAAC
 CGTGCTGTCA ACTCTCTGCA GAATGCATCA GCAATTGAGA GCATTCTTAA
 AAATCTCCTG CCATGTCTGC CCCTGGCCAC GGCCGCACCC ACGCGACATC
 CAATCCATAT CAAGGACGGT GACTGGAATG AATTCCGTCG TAAACTGACC
 TTCTATCTGA AAACCTTGA GAACGCGCAG GCTCAACAG

20 [SEQ ID NO:408]

Examples 69-76

The variants in Table 5 were constructed by cassette
 25 mutagenesis using methods described in the Materials
 and Methods and the Examples contained herein,
 particularly Examples 54-58 . Parental plasmid DNA
 (Table 5), digested with the appropriate restriction
 enzymes (Table 5), was ligated with the indicated
 30 annealed pairs of complementary oligonucleotides (Table
 5). The assembled oligonucleotides create appropriate
 restriction ends and a portion of the (15-125) hIL-3
 gene sequence (pMON13288 [SEQ ID NO:100]). Individual
 isolates were screened by restriction analysis and DNA
 35 sequenced to confirm that the desired changes in the
 (15-125) hIL-3 variant gene were made. The

oligonucleotides create change(s) in the (15-125) hIL-3 gene which encode the corresponding amino acid substitution(s) in the variant polypeptide (Table 5). The amino acids substitutions in addition to and/or
5 different from those in polypeptide # 25 [SEQ ID NO:89] are indicated in Table 5. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and
10 the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 5 is shown in Table 1.

Examples 77-82

15 The variants in Table 6 were constructed by methods described in the Materials and Methods and the Examples contained herein, particularly in Examples 60 and 61. Parental plasmid DNA (Table 6), digested with the
20 appropriate restriction enzymes (Table 6), was ligated with the indicated restriction fragment (Table 6). Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made.
25 The resulting mutated (15-125) hIL-3 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 6). The amino acids substitutions in addition to and/or different from those in polypeptide # 25 [SEQ ID NO:89] are indicated in Table
30 6. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells)
35 for some of the variants in Table 6 is shown in Table 1.

Example 83

Construction of pMON13368

5 One of the DNA fragments to construct the plasmid, pMON13368, was generated by site-directed mutagenesis employing PCR techniques described in the Materials and Methods and the Examples contained herein, particularly Example 53. The template for the PCR reaction was plasmid, pMON13289, DNA using the oligonucleotides, Oligo #B13 18I23A25H [SEQ ID NO: 182] and Oligo #B14 2341HIN3 [SEQ ID NO:183], as primers. The resulting DNA product was digested with the restriction enzymes NcoI and HindIII. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The restriction fragment was purified by phenol/chloroform extraction and precipitation with equal volume isopropanol in the presence of 2M NH₄OAc. The oligonucleotide, Oligo #B13 18I23A25H [SEQ ID NO:182], changes the codon at position 23 of (15-125) hIL-3 variant gene pMON13289 [SEQ ID NO:103] from 'ATT' to 'GCA' (Ile to Ala). The 3619 base pair NcoI, HindIII restriction fragment from pMON2341 was ligated to the PCR-generated NcoI, HindIII restriction fragment. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The plasmid, pMON13368, contains the (15-125) hIL-3 variant gene (DNA sequence #B15 [SEQ ID NO:346]) which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B15 [SEQ ID NO.:278]

35

Example 84

Construction of pMON13380

Plasmid, pMON13368, DNA was digested with restriction
5 enzymes EcoRI and HindIII. The resulting 3900 base pair
EcoRI,HindIII fragment contains the following genetic
elements; beta-lactamase gene (AMP), pBR327 origin of
replication, phage F1 origin of replication as the
transcription terminator, precA promoter, gl0L ribosome
10 binding site and the DNA sequence encoding amino acids
15-105 of the variant pMON13368. The 3900 base pair
EcoRI,HindIII restriction fragment from pMON13368 was
ligated to the following annealed complementary
oligonucleotides.

15
Oligo # B48 9E12Q6V1 [SEQ ID NO:217]
Oligo # B49 9E12Q6V3 [SEQ ID NO:218]

Oligo #49 120Q123E2 [SEQ ID NO:63]
20 Oligo #50 120Q123E4 [SEQ ID NO:64]

When assembled, the oligonucleotides create EcoRI and
HindIII restriction ends and the DNA sequence that
encodes amino acids 106-125 of (15-125) hIL-3 with the
25 following amino acid substitution; 109E, 112Q, 116V,
120Q and 123E. The codons used in the (15-125) hIL-3
gene are those found in the hIL-3 cDNA sequence except
at those positions where amino acid substitutions were
made. Individual isolates were screened by restriction
30 analysis and DNA sequenced to confirm that the desired
changes in the (15-125) hIL-3 variant gene were made.
The plasmid, pMON13380, contains the (15-125) hIL-3
variant gene (DNA sequence #B16 [SEQ ID NO:347]) which
encodes the (15-125) hIL-3 variant polypeptide with the
35 following amino acid sequence:

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Polypeptide #B16 [SEQ ID NO.:279]

Example 85

5 Construction of pMON13476

One of the DNA fragments to construct the plasmid, pMON13476, was generated by site-directed mutagenesis employing PCR techniques described in the Materials and
10 Methods and the Examples contained herein, particularly Example 54. The template for the PCR reaction was plasmid, pMON13287, DNA using the oligonucleotides, Oligo #B13 18I23A25H [SEQ ID NO:182] and Oligo #B14 2341HIN3 [SEQ ID NO.:183] as primers.
15 The resulting DNA product was digested with the restriction enzymes NcoI and HindIII. Upon completion, the digest was heated at 70°C for 15 minutes to inactivate the enzymes. The restriction fragment was purified by phenol/chloroform extraction and
20 precipitation with equal volume isopropanol in the presence of 2M NH₄OAc. The oligonucleotide, Oligo #B13 18I23A25H [SEQ ID NO.:182], changes the codon at position 23 of (15-125) hIL-3 variant gene, pMON13287, [SEQ ID NO:97] from 'ATT' to 'GCA' (Ile to Ala). The
25 3619 base pair NcoI, HindIII restriction fragment from pMON2341 was ligated to the PCR-generated NcoI, HindIII restriction fragment. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant
30 gene were made. The resulting clone also contained a change, that was not designed in the mutagenic oligonucleotide, which changed the codon at position -1 from 'GCT' to 'GAT' which changes the amino acid from Alanine to Aspartic Acid. The plasmid, pMON13476,
35 contains the (15-125) hIL-3 variant gene (DNA sequence #B52 [SEQ ID NO:303]) which encodes the (15-125) hIL-3

variant polypeptide with the following amino acid sequence:

Polypeptide #B52 [SEQ ID NO.:314]

5

Examples 86-92

The variants in Table 7 were constructed by PCR techniques using methods described in the Materials and Methods and the Example contained herein, particularly Example 51. Two sequential PCR reactions were used to create the variants. In the first PCR reaction pMON13287 plasmid DNA served as the template and the two oligonucleotides indicated in Table 7 served as the primers. Following the PCR extension reaction, the PCR product was partially purified to remove primer that was not extended. In the second PCR reaction pMON13287 plasmid DNA served as the template, the purified PCR product from the first PCR reaction served as one of the primers and the Oligo #B14 2341Hin3 [SEQ ID NO:183] as the second primer. The product from the second PCR reaction was partially purified and digested with restriction enzymes NcoI and HindIII and ligated with the 3619 base pair NcoI,HindIII fragment from pMON2341. Individual isolates were screened by restriction analysis and DNA sequenced to confirm that the desired changes in the (15-125) hIL-3 variant gene were made. The amino acids substitutions in addition to and/or different from those in polypeptide # 24 [SEQ ID NO:88] are indicated in Table 7. The table also shows the plasmid designation (pMON number), DNA sequence identification number for the mutated hIL-3 gene and the identification number for the the resulting variant polypeptide. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 7 is shown in Table 1.

Examples 93-120

The variants in Table 8 were constructed by cassette
5 mutagenesis using methods described in the Materials
and Methods and the Examples contained here,
particularly Examples 54-58. Parental plasmid DNA
(Table 8), digested with the appropriate restriction
enzymes (Table 8), was ligated with the indicated
10 annealed pairs of complementary oligonucleotides (Table
8). The assembled oligonucleotides create the
appropriate restriction ends and a portion of (15-125)
hIL-3 gene (pMON13288 [SEQ ID NO:100]) sequence. The
oligonucleotides create change(s) in the (15-125) hIL-3
15 variant gene which encode the corresponding amino acid
substitution(s); and/or deletions from the C-terminus
of the variant polypeptide (Table 8). Individual
isolates were screened by restriction analysis and DNA
sequenced to confirm that the desired changes in the
20 (15-125) hIL-3 variant gene were made. The amino acids
substitutions in addition to and/or different from
those in polypeptide # 25 [SEQ ID NO:88] are indicated
in Table 8. The table also shows the plasmid
designation (pMON number), DNA sequence identification
25 number for the mutated hIL-3 gene and the
identification number for the the resulting variant
polypeptide. The biological activity (growth promoting
activity in AML 193 cells) for some of the variants in
Table 5 is shown in Table 1.
30

Example 121

Construction of pMON13446

Plasmid, pMON13287, DNA (purified from the E. coli
35 strain GM48 {dam-}) was digested with restriction
enzymes NcoI and ClaI. The resulting 3942 base pair
NcoI, ClaI fragment contains the following genetic

elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the transcription terminator, precA promoter, gl0L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant pMON13287. The 3942 base pair NcoI,ClaI restriction fragment from pMON13368 was ligated to the following annealed complementary oligonucleotides.

10 Oligo #B57 338UP [SEQ ID NO:226]

Oligo #B56 338DOWN [SEQ ID NO:225]

When assembled, the oligonucleotides create NcoI and
15 ClaI restriction ends and the DNA sequence that encodes
the following 14 amino acid sequence; Met Ala Tyr Pro
Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403]
and the DNA sequence which encodes amino acids 15-20 of
the (15-125) hIL-3 variant gene, pMON13287 [SEQ ID
20 NO:97]. The resulting variant polypeptide has a 14
amino acid N-terminal extension fused to the (15-125)
hIL-3 variant polypeptide, pMON13288 [SEQ ID NO: 88].
The plasmid, pMON13446, contains the (15-125) hIL-3
variant gene (DNA sequence #B53 [SEQ ID NO:404]) which
25 encodes the (15-125) hIL-3 variant polypeptide with the
following amino acid sequence:

Polypeptide #B53 [SEQ ID NO.:315]

30 Example B54

Construction of pMON13390

Plasmid, pMON13288, DNA (purified from the E. coli strain GM48 {dam-}) was digested with restriction enzymes NcoI and ClaI. The resulting 3942 base pair NcoI,ClaI fragment contains the following genetic elements; beta-lactamase gene (AMP), pBR327 origin of replication, phage F1 origin of replication as the

transcription terminator, precA promoter, gl0L ribosome binding site and the DNA sequence encoding amino acids 21-125 of the (15-125) hIL-3 variant pMON13288. The 3942 base pair NcoI, ClaI restriction fragment from pMON13288 was ligated to the following annealed complementary oligonucleotides.

| | | | |
|----|------------|---------|-----------------|
| | Oligo #B57 | 338UP | [SEQ ID NO:226] |
| 10 | Oligo #B56 | 338DOWN | [SEQ ID NO:225] |

When assembled, the oligonucleotides create NcoI and ClaI restriction ends and the DNA sequence which encodes the following 14 amino acid sequence; Met Ala Tyr Pro Glu Thr Asp Tyr Lys Asp Asp Asp Asp Lys [SEQ ID NO:403] and the DNA sequence which encodes amino acids 15-20 of the (15-125) hIL-3 variant gene pMON13288 [SEQ ID NO:100]. The resulting variant has a 14 amino acid N-terminal extension fused to the (15-125) hIL-3 variant polypeptide, pMON13288 [SEQ ID NO:88]. The plasmid, pMON13390, contains the (15-125) hIL-3 variant gene (DNA sequence #B54 [SEQ ID NO.:405] which encodes the (15-125) hIL-3 variant polypeptide with the following amino acid sequence:

Polypeptide #B54 [SEQ ID NO:316]

Examples 133-136

The variants in Table 10 were constructed by methods described in Materials and Methods and in Examples contained herein, particularly Examples 54-58. Parental plasmid DNA (Table 10), digested with the appropriate restriction enzymes (Table 10) was ligated with the indicated restriction fragment containing the changes listed (Table 10). The resulting mutated (15-125) IL-3 genes encode the corresponding amino acid substitutions in the variant polypeptides (Table 10). The amino acid

substitutions in addition to and/or different from those in polypeptide #25 [SEQ ID NO: 89] are indicated in Table 10. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 10 is shown in Table 1.

Examples 123-132

The variants in Table 9 were constructed by cassette mutagenesis using methods described in Materials and Methods and in Examples 54-58 contained herein. Parental plasmid DNA (Table 9), digested with the appropriate restriction enzymes (Table 9), was ligated with the indicated annealed pairs of complementary oligonucleoties (Table 9). The assembled oligonucleotides create the appropriate restriction fragment which was inserted into the (15-125) hIL-3 gene (pMON13288 [SEQ ID NO:100] between these restriction sites. The deletions or substitutions encoded by the oligonucleotide in the (15-125) IL-3 gene correspond to the amino acid deletions or substitutions in the variant polypeptide (Table 9). The amino acid substitutions or deletions, in addition to and/or different from those in the polypeptide #25 [SEQ ID NO:89] are indicated in Table 9. The biological activity (growth promoting activity in AML 193 cells) for some of the variants in Table 9 is shown in Table 1.

Formula XI shown below is a representation of a [(15-125)hIL-3 mutein] with numbers in bold type added above the amino acids to represent the position at which the amino acid below the bolded number appears in native (1-133)hIL-3 [e. g. the amino acid at position 1 of Formula XI corresponds to the Asn which appears at position 15 in native (1-133)hIL-3]. The number shown in bold indicates the amino acids that correspond to

the native IL-3(1-133). The non-bold members below the amino acids sequences are for Seq Id reference numbers. When the muteins are expressed the initial amino acid may be preceded by Met- or Met-Ala-.

[illegible]

Table 5

| Example | pMON number | Parental plasmid/ restriction digest | oligo pair 1,4 | oligo pair 2,5 | oligo pair 3,6 | amino acid changes | resulting polypeptide |
|------------|----------------------------|---|--|--|--|-----------------------|---------------------------------|
| Example 69 | pMON13406 SEQ ID NO:332 | pMON13288/ NcoI, EcoRV | 19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171 | 29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20 | 42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26 | 19Ala | polypeptide B1 SEQ ID NO:264 |
| Example 70 | pMON13414 SEQ ID NO:333 | pMON13288/ NcoI, EcoRV | 19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173 | 29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20 | 42S45M3 OLIGO# 11 SEQ ID NO:25 42S45M6 OLIGO# 12 SEQ ID NO:26 | 19Ile | polypeptide B2 SEQ ID NO:265 |
| Example 71 | pMON13407 SEQ ID NO:334 | pMON13288/ NcoI, EcoRV | 18I25H1 OLIGO# 1 SEQ ID NO:15 18I25H4 OLIGO# 2 SEQ ID NO:16 | 29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20 | 42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181 | 45Val | polypeptide B3 SEQ ID NO:266 |
| Example 72 | pMON13405 SEQ ID NO:335 | pMON13288/ NcoI, EcoRV | 19Ala1 OLIGO# B1 SEQ ID NO:170 19Ala4 OLIGO# B2 SEQ ID NO:171 | 29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20 | 42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181 | 19Ala,45Val | polypeptide B4 SEQ ID NO:267 |
| Example 73 | pMON13415 SEQ ID NO:336 | pMON13288/ NcoI, EcoRV | 19Ile1 OLIGO# B3 SEQ ID NO:172 19Ile4 OLIGO# B4 SEQ ID NO:173 | 29R32N37P2 OLIGO# 5 SEQ ID NO:19 29R32N37P5 OLIGO# 6 SEQ ID NO:20 | 42S45V3 OLIGO# B11 SEQ ID NO:180 42S45V6 OLIGO# B12 SEQ ID NO:181 | 19Ile,45Val | polypeptide B5 SEQ ID NO:268 |
| Example 74 | pMON13408 SEQ ID NO:337 | pMON13288/ EcoRV, NsiI | 49Ile1 OLIGO# B7 SEQ ID NO:176 49Ile4 OLIGO# B8 SEQ ID NO:177 | 59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40 | 67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44 | 49Ile | polypeptide B6 SEQ ID NO:269 |

Table 5 cont

| | | | | | | | |
|------------|----------------------------|---------------------------|---|--|--|-------|---------------------------------|
| Example 75 | pMON13409 SEQ ID NO:338 | pMON13288/ EcoRV, NotI | 49Leu1 SEQ ID NO:178 OLIGO# B9 49Leu4 OLIGO# B10 SEQ ID NO:179 | 59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40 | 67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44 | 49Leu | polypeptide B7 SEQ ID NO:270 |
| Example 76 | pMON13410 SEQ ID NO:339 | pMON13288/ EcoRV, NotI | 49Asp1 OLIGO# B5 SEQ ID NO:174 49Asp4 OLIGO# B6 SEQ ID NO:176 | 59L62V2 OLIGO# 25 SEQ ID NO:39 59L62V5 OLIGO# 26 SEQ ID NO:40 | 67H69E3 OLIGO# 29 SEQ ID NO:43 67H69E6 OLIGO# 30 SEQ ID NO:44 | 49Asp | polypeptide B8 SEQ ID NO:271 |

Table 6

| Example No | plasmid pMON number | Parental plasmid/ restriction digest | restriction fragment | amino acid substitutions | resulting polypeptide |
|------------|----------------------------|---|---|---------------------------|----------------------------------|
| Example 77 | pMON13422 SEQ ID NO:340 | pMON13408/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13405 | 19Ala, 45Val, 49Ple | polypeptide B9 SEQ ID NO:272 |
| Example 78 | pMON13423 SEQ ID NO:341 | pMON13408/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13415 | 19Ile, 45Val, 49Ple | polypeptide B10 SEQ ID NO:273 |
| Example 79 | pMON13424 SEQ ID NO:342 | pMON13409/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13405 | 19Ala, 45Val, 49Leu | polypeptide B11 SEQ ID NO:274 |
| Example 80 | pMON13425 SEQ ID NO:343 | pMON13409/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13415 | 19Ile, 45Val, 49Leu | polypeptide B12 SEQ ID NO:275 |
| Example 81 | pMON13426 SEQ ID NO:344 | pMON13410/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13405 | 19Ala, 45Val, 49Asp | polypeptide B13 SEQ ID NO:276 |
| Example 82 | pMON13429 SEQ ID NO:345 | pMON13410/ NcoI, EcoRV | 99 base pair NcoI, EcoRV fragment from pMON13415 | 19Ile, 45Val, 49Asp | polypeptide B14 SEQ ID NO:277 |

Table 7

| Example | pMON number | template | Step one PCR primer1 | Step one PCR primer2 | Step two PCR primer1 product from step one | Step two PCR primer2 OLIGO# B14 SEQ ID NO:183 | Amino Acid Substitutions | Polypeptide |
|------------|----------------------------|-----------|---|---|---|--|-----------------------------|---------------------------------------|
| Example 86 | pMON13475 SEQ ID NO:348 | pMON13287 | 1823A25H OLIGO# B13 SEQ ID NO:182 | 42D45V46S50D OLIGO# B19 SEQ ID NO:188 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 42D,46S,50D | Polypeptide # B17 SEQ ID NO 280 |
| Example 87 | pMON13366 SEQ ID NO:349 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42D45V46S50D OLIGO# B19 SEQ ID NO:188 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 42N,46S,50D | Polypeptide # B18 SEQ ID NO 281 |
| Example 88 | pMON13367 SEQ ID NO:350 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42A45V46S50D OLIGO# B17 SEQ ID NO:186 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 46S,50D | Polypeptide # B19 SEQ ID NO 282 |
| Example 89 | pMON13369 SEQ ID NO:351 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42D45V46S50D OLIGO# B21 SEQ ID NO:190 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 42D,46S,50D | Polypeptide # B20 SEQ ID NO 283 |
| Example 90 | pMON13370 SEQ ID NO:352 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42A45M46S50D OLIGO# B16 SEQ ID NO:185 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 45M,46S,50D | Polypeptide # B21 SEQ ID NO 284 |
| Example 91 | pMON13373 SEQ ID NO:353 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42D45M46S50D OLIGO# B18 SEQ ID NO:187 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 42D,45M,46S 50D | Polypeptide # B22 SEQ ID NO 285 |
| Example 92 | pMON13374 SEQ ID NO:354 | pMON13287 | 2341NCO OLIGO# B15 SEQ ID NO:184 | 42S45M46S50D OLIGO# B20 SEQ ID NO:189 | product from step one | 2341HIN3 OLIGO# B14 SEQ ID NO:183 | 42S,45M46S 50D | Polypeptide # B23 SEQ ID NO 286 |

| Example | plasmid | patental Plasmid | oligo pair | oligo pair | oligo pair | oligo pair | resulting amino acid sub(s). | polypeptide |
|------------|----------------------------|------------------------------|--|--|--|------------|-------------------------------------|----------------------------------|
| Example 93 | PHON13375 SEQ ID NO:355 | PHON13287/ EcoRI, HindIII | S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 SECRI033 OLIGO# B51 OLIGO# B53 SEQ ID NO:220 | S116VD31 OLIGO# B52 SEQ ID NO:221 SECRI033 OLIGO# B53 SEQ ID NO:222 | | | 15-119 | polypeptide B24 SEQ ID NO:287 |
| Example 94 | PHON13376 SEQ ID NO:356 | PHON13476/ EcoRI, HindIII | S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 SECRI033 OLIGO# B55 SEQ ID NO:224 | S116VD31 OLIGO# B52 SEQ ID NO:221 SECRI033 OLIGO# B53 SEQ ID NO:222 | | | 15-119, 23A, 112Q | polypeptide B25 SEQ ID NO:288 |
| Example 95 | PHON13377 SEQ ID NO:357 | PHON13475/ EcoRI, HindIII | S9E2Q6V1 OLIGO# B54 SEQ ID NO:223 S9E2Q6V3 SECRI033 OLIGO# B55 SEQ ID NO:224 | S116VD31 OLIGO# B52 SEQ ID NO:221 SECRI033 OLIGO# B53 SEQ ID NO:222 | | | 15-119, 23A, 42D, 46S, 50D, 112Q | polypeptide B26 SEQ ID NO:289 |
| Example 96 | PHON13378 SEQ ID NO:358 | PHON13365/ EcoRI, HindIII | S09E16V1 OLIGO# B50 SEQ ID NO:219 S09E16V3 SECRI033 OLIGO# B51 OLIGO# B53 SEQ ID NO:220 | S116VD31 OLIGO# B52 SEQ ID NO:221 SECRI033 OLIGO# B53 SEQ ID NO:222 | | | 15-119, 23A | polypeptide B27 SEQ ID NO:290 |
| Example 97 | PHON13379 SEQ ID NO:359 | PHON13367/ EcoRI, HindIII | S8L2Q6V1 OLIGO# B48 SEQ ID NO:217 S8L2Q6V3 OLIGO# B49 OLIGO# B51 SEQ ID NO:220 | 12Q0I23E2 OLIGO# 49 SEQ ID NO:63 12Q0I23E4 OLIGO# 50 SEQ ID NO:64 | | | 46S, 50D, 112Q | polypeptide B28 SEQ ID NO:291 |
| Example 98 | PHON13385 SEQ ID NO:360 | PHON13287/ NcoI, EcoRV | 181ZSH1 OLIGO#1 SEQ ID NO:15 181ZSH4 OLIGO#2 SEQ ID NO:16 | 29VJ2R34S2 OLIGO# B28 SEQ ID NO:197 29VJ2R34S5 OLIGO# B29 SEQ ID NO:198 | 42A4SV3 OLIGO# 9 SEQ ID NO:23 42A4SV6 OLIGO# 10 SEQ ID NO:24 | | 29V, 32R, 34S | polypeptide B29 SEQ ID NO:292 |
| Example 99 | PHON13381 SEQ ID NO:361 | PHON13287/ HaeII, EcoRI | 73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56 | 82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214 | 101A10S04 OLIGO# 43 SEQ ID NO:49 101A10S08 OLIGO# 44 SEQ ID NO:50 | 82W | | polypeptide B30 SEQ ID NO:293 |

Table 8 cont

| | | | | | | | | |
|-------------|----------------------------|------------------------------|--|--|--|--|----------------------------|----------------------------------|
| Example 100 | PHONI3383 SEQ ID NO:362 | PHONI3475/ EcoRI, HindIII | 9E1206V1 OLIGO# B48 SEQ ID NO:217 9E1206V5 OLIGO# B49 SEQ ID NO:218 | 1200123E2 OLIGO# 49 SEQ ID NO:63 1200123E4 OLIGO# 50 SEQ ID NO:64 | | | 23A, 42D, 46S, 50D 1120 | polypeptide B31 SEQ ID NO:294 |
| Example 101 | PHONI3384 SEQ ID NO:363 | PHONI3287/ EcoRI, HindIII | 9E1206V1 OLIGO# B48 SEQ ID NO:217 9E1206V5 OLIGO# B49 SEQ ID NO:218 | 1200123E2 OLIGO# 49 SEQ ID NO:63 1200123E4 OLIGO# 50 SEQ ID NO:64 | | | 1120 | polypeptide B32 SEQ ID NO:295 |
| Example 102 | PHONI3388 SEQ ID NO:364 | PHONI3287/ EcoRV, NsiI | 50D36S1 OLIGO# B42 SEQ ID NO:211 50A3P4 OLIGO# B41 SEQ ID NO:210 | 60S62V2 OLIGO# 27 SEQ ID NO:41 56SERS OLIGO# B43 SEQ ID NO:212 | 67N69E3 OLIGO# 31 SEQ ID NO:45 67N69E6 OLIGO# 32 SEQ ID NO:46 | | 50D, 56S | polypeptide B33 SEQ ID NO:296 |
| Example 103 | PHONI3389 SEQ ID NO:365 | PHONI3287/ NcoI, EcoRV | 18125H1 OLIGO# 1 SEQ ID NO:15 18125H4 OLIGO# 4 SEQ ID NO:16 | 29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18 | 42D45M3 OLIGO# B32 SEQ ID NO:201 42D45M6 OLIGO# B33 SEQ ID NO:202 | | 42D, 45M | polypeptide B34 SEQ ID NO:297 |
| Example 104 | PHONI3391 SEQ ID NO:366 | PHONI3287/ NcoI, EcoRV | 18125H1 OLIGO# 1 SEQ ID NO:15 18125H4 OLIGO# 2 SEQ ID NO:16 | 34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200 | 42A4SV3 OLIGO# 9 SEQ ID NO:23 42A4SV6 OLIGO# 10 SEQ ID NO:24 | | 34S | polypeptide B35 SEQ ID NO:298 |
| Example 105 | PHONI3392 SEQ ID NO:367 | PHONI3287/ NcoI, EcoRV | 18125H1 OLIGO# 1 SEQ ID NO:15 18125H4 OLIGO# 2 SEQ ID NO:16 | 29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18 | 42D45V3 OLIGO# B34 SEQ ID NO:203 42D45V6 OLIGO# B35 SEQ ID NO:204 | | 42D | polypeptide B36 SEQ ID NO:299 |
| Example 106 | PHONI3393 SEQ ID NO:368 | PHONI3287/ NcoI, EcoRV | 23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196 | 34SER1 OLIGO# B30 SEQ ID NO:199 34SER5 OLIGO# B31 SEQ ID NO:200 | 42D45M46S3 OLIGO# B36 SEQ ID NO:205 42D45M46S6 OLIGO# B37 SEQ ID NO:206 | | 23A, 34S, 42D, 45M 46S | polypeptide B37 SEQ ID NO:300 |

Table 8 cont

| | | | | | | | | |
|-------------|----------------------------|------------------------------|--|--|--|--|--------------------------------|----------------------------------|
| Example 107 | PHONI3394 SEQ ID NO:369 | PHONI3287/ NcoI, EcoRV | 18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16 | 29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18 | 42D45V46S3 OLIGO# B36 SEQ ID NO:205 42D45V46S6 OLIGO# B37 SEQ ID NO:206 | | 42D, 45H, 46S | polypeptide B38 SEQ ID NO:301 |
| Example 108 | PHONI3395 SEQ ID NO:370 | PHONI3287/ NcoI, EcoRV | 23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196 | 29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198 | 42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208 | | 23A, 29V, 32R, 34S 42D, 46S | polypeptide B39 SEQ ID NO:302 |
| Example 109 | PHONI3396 SEQ ID NO:371 | PHONI3287/ NcoI, EcoRI | 73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56 | 79R8202 OLIGO# 39 SEQ ID NO:53 79R8205 OLIGO# 40 SEQ ID NO:54 | 100ARG3 SEQ ID NO: 87S93S98I7 OLIGO# 36 SEQ ID NO:50 | 100MET4 OLIGO# B24 SEQ ID NO:193 10R01H8 OLIGO# B25 SEQ ID NO:194 | 100R, 101H | polypeptide B40 SEQ ID NO:303 |
| Example 110 | PHONI3397 SEQ ID NO:372 | PHONI3287/ NcoI, EcoRI | 73G76A1 OLIGO# 41 SEQ ID NO:55 73G76A4 OLIGO# 42 SEQ ID NO:56 | 82TRP2 OLIGO# B44 SEQ ID NO:213 82TRP5 OLIGO# B45 SEQ ID NO:214 | 100ARG3 OLIGO# B22 SEQ ID NO:191 87S93S98I7 OLIGO# 36 SEQ ID NO:50 | 100MET4 OLIGO# B24 SEQ ID NO:193 10R01H8 OLIGO# B25 SEQ ID NO:194 | 82H, 100R, 101H | polypeptide B41 SEQ ID NO:304 |
| Example 111 | PHONI3398 SEQ ID NO:373 | PHONI3287/ NcoI, EcoRV | 18125H1 OLIGO#1 SEQ ID NO:15 18125H4 OLIGO#2 SEQ ID NO:16 | 29R32A37P2 OLIGO# 3 SEQ ID NO:17 29R32A37P5 OLIGO# 4 SEQ ID NO:18 | 42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208 | | 42D, 46S | polypeptide B42 SEQ ID NO:305 |
| Example 112 | PHONI3399 SEQ ID NO:374 | PHONI3368/ NcoI, EcoRV | 23ALA1 OLIGO# B26 SEQ ID NO:195 23ALA4 OLIGO# B27 SEQ ID NO:196 | 29V32R34S2 OLIGO# B28 SEQ ID NO:197 29V32R34S5 OLIGO# B29 SEQ ID NO:198 | 42D45V46S3 OLIGO# B38 SEQ ID NO:207 42D45V46S6 OLIGO# B39 SEQ ID NO:208 | | 23A, 29V, 32R, 34S 42D, 46S | polypeptide B43 SEQ ID NO:306 |
| Example 113 | PHONI3404 SEQ ID NO:375 | PHONI3287/ EcoRI, HindIII | S9E206V1 OLIGO# B54 SEQ ID NO:223 S9E206V3 OLIGO# B55 SEQ ID NO:224 | S116V031 OLIGO# B52 SEQ ID NO:221 S9E206V3 OLIGO# B53 SEQ ID NO:222 | | | 15-119 1170 | polypeptide B44 SEQ ID NO:307 |

Table 8 cont

| | | | | | | | |
|-------------|----------------------------|------------------------------|---|--|--|--------------------------------------|----------------------------------|
| Example 114 | PHON13387 SEQ ID NO:376 | PHON13387/ EcoRV, NsiI | 50ASP1 OLIGO1 B40 SEQ ID NO:309 50ASP4 OLIGO1 B41 SEQ ID NO:210 | 60S62V2 OLIGO1 27 SEQ ID NO:41 60S62V5 OLIGO1 28 SEQ ID NO:42 | 67N69E3 OLIGO1 31 SEQ ID NO:45 67N69E6 OLIGO1 32 SEQ ID NO:46 | 50D | polypeptide B45 SEQ ID NO:308 |
| Example 115 | PHON13416 SEQ ID NO:377 | PHON13387/ NcoI/EcoRV | 1812SH1 OLIGO1 3 SEQ ID NO:15 1812SH4 OLIGO1 4 SEQ ID NO:16 | 29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18 | 42D45M653 OLIGO1 B38 SEQ ID NO:207 42D45M656 OLIGO1 B39 SEQ ID NO:208 | 42D, 46S, 50D | polypeptide B46 SEQ ID NO:309 |
| Example 116 | PHON13417 SEQ ID NO:378 | PHON13387/ NcoI/EcoRV | 1812SH1 OLIGO1 3 SEQ ID NO:15 1812SH4 OLIGO1 4 SEQ ID NO:16 | 29R32A37P2 OLIGO1 3 SEQ ID NO:17 29R32A37P5 OLIGO1 4 SEQ ID NO:18 | 42D45M653 OLIGO1 B36 SEQ ID NO:205 42D45M656 OLIGO1 B37 SEQ ID NO:206 | 42D, 45M, 46S, 50D | polypeptide B47 SEQ ID NO:310 |
| Example 117 | PHON13420 SEQ ID NO:379 | PHON13388/ NcoI, EcoRV | 23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196 | 34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200 | 42D45M653 OLIGO1 B38 SEQ ID NO:207 42D45M656 OLIGO1 B39 SEQ ID NO:208 | 23A, 34S, 42D, 46S, 50D, 56S | polypeptide B48 SEQ ID NO:311 |
| Example 118 | PHON13421 SEQ ID NO:380 | PHON13388/ NcoI, EcoRV | 23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196 | 34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200 | 42D45M653 OLIGO1 B36 SEQ ID NO:205 42D45M656 OLIGO1 B37 SEQ ID NO:206 | 23A, 34S, 42D, 45M, 46S, 50D, 56S | polypeptide B49 SEQ ID NO:331 |
| Example 119 | PHON13432 SEQ ID NO:381 | PHON13387/ NcoI, EcoRV | 23ALA1 OLIGO1 B26 SEQ ID NO:195 23ALA4 OLIGO1 B27 SEQ ID NO:196 | 34SER1 OLIGO1 B30 SEQ ID NO:199 34SER5 OLIGO1 B31 SEQ ID NO:200 | 42D45M653 OLIGO1 B36 SEQ ID NO:205 42D45M656 OLIGO1 B37 SEQ ID NO:206 | 23A, 34S, 42D, 45M, 46S, 50D | polypeptide B50 SEQ ID NO:312 |
| Example 120 | PHON13382 SEQ ID NO:382 | PHON13387/ EcoRI, HindIII | 9E12Q6W1 OLIGO1 B46 SEQ ID NO:215 9E12Q16W3 OLIGO1 B47 SEQ ID NO:216 | 12Q0123E2 OLIGO1 49 SEQ ID NO:63 12Q0123E4 OLIGO1 50 SEQ ID NO:64 | | 112Q, 116W | polypeptide B51 SEQ ID NO:313 |

Table 9

| Example No. | Plasmid | Parental Plasmid/ Restriction Digest | Oligo pair | Oligo pair | Oligo pair | Amino acid changes | Polypeptide |
|-------------|----------------------------|---|--|--|--|---|-----------------------------------|
| Example 124 | pMON13400 SEQ ID NO:384 | pMON13288 Restriction NcoI-EcoRV | 20P23A1 SEQ ID NO:232 20P23A4 SEQ ID NO:233 | 2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237 | 38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239 | 20P 23A 291 34S 37S 38A 45V 46S | Polypeptide C-2 SEQ ID NO:317 |
| Example 125 | pMON13402 SEQ ID NO:385 | pMON13288 Restriction NcoI-EcoRV | 23L1 SEQ ID NO:234 23L4 SEQ ID NO:235 | 2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237 | 38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239 | 23L 291 34S 37S 38A 45V 46S | Polypeptide C-3 SEQ ID NO:318 |
| Example 131 | pMON13440 SEQ ID NO:386 | pMON13288 Restriction NcoI-EcoRV | 1813A5H1 SEQ ID NO:195 1813A5I4 SEQ ID NO:196 | 2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237 | 38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239 | 181 23A 25H 291 34S 37S 38A 45V 46S | Polypeptide C-10 SEQ ID NO:319 |
| Example 132 | pMON13451 SEQ ID NO:387 | pMON13288 Restriction NcoI-EcoRV | 1910L3A1 SEQ ID NO:230 1910L3A4 SEQ ID NO:231 | 2914S7S2 SEQ ID NO:236 2914S7S5 SEQ ID NO:237 | 38A5V6S3 SEQ ID NO:238 38A5V6S3 SEQ ID NO:239 | 191 20L 23A 291 34S 37S 38A 45V 46S | Polypeptide C-11 SEQ ID NO:320 |
| Example 130 | pMON13419 SEQ ID NO:389 | pMON13288 Restriction EcoRV-NaII | 60D61S1 SEQ ID NO:240 60D61S4 SEQ ID NO:241 | 62P3H5S2 SEQ ID NO:244 62P3H5 SEQ ID NO:246 | 67Q3 SEQ ID NO:248 65S67Q6 SEQ ID NO:247 | 60D 61S 62P 63H 65S 67Q | Polypeptide C-8 SEQ ID NO:325 |
| Example 126 | pMON13403 SEQ ID NO:388 | pMON13288 Restriction EcoRV-NaII | 60D61S1 SEQ ID NO:240 60D61S4 SEQ ID NO:241 | 62P3H2 SEQ ID NO:245 62P3H5 SEQ ID NO:246 | 67Q3 SEQ ID NO:248 67Q6 SEQ ID NO:249 | 60D 61S 62P 63H 67Q | Polypeptide C-4 SEQ ID NO:321 |

Table 9 cont

| | | | | | | | | |
|-------------|----------------------------|---|--|--|--|--|--|----------------------------------|
| Example 123 | pMON13418 SEQ ID NO:393 | pMON13288 Restriction NotI-EcoRI | 76P1 SEQ ID NO:250 76P5 SEQ ID NO:251 | 78S2 SEQ ID NO:252 78S6 SEQ ID NO:253 | 6VYWPTT3 SEQ ID NO:242 6VYWPTT7 SEQ ID NO:243 | 10LA105Q4 SEQ ID NO:57 10LA105Q8 SEQ ID NO:58 | 76P 78S 85V 87Y 88W 91P 85T 98T | Polypeptide C-1 SEQ ID NO:326 |
| Example 127 | pMON13411 SEQ ID NO:390 | pMON13288 Restriction EcoRI-HindIII | 09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228 | 120Q123E2 SEQ ID NO:63 120Q123E4 SEQ ID NO:64 | | | 109L 112Q 116S | Polypeptide C-5 SEQ ID NO:322 |
| Example 128 | pMON13412 SEQ ID NO:391 | pMON13288 Restriction EcoRI-HindIII | 9LQ51181 Seq ID NO:255 9LQ51183 SEQ ID NO:256 | | | | 15-118 109L 112Q 116S | Polypeptide C-6 SEQ ID NO:323 |
| Example 129 | pMON13413 SEQ ID NO:392 | pMON13288 Restriction EcoRI-HindIII | 09L2Q6S1 Seq ID NO:227 09L2Q6S3 SEQ ID NO:228 | 117S2 SEQ ID NO:229 120Q123E4 SEQ ID NO:64 | | | 109L 112Q 116S 117S | Polypeptide C-7 SEQ ID NO:324 |

Table 10

| Example No | Plasmid | Parental plasmid/ Restriction digest | Restriction fragment | Amino Acid changes | Polypeptide |
|-------------|-------------------------------|---|--|---|---|
| Example 133 | pMON13428 SEQ ID NO:394 | pMON13411 NsiI-EcoRI | 102 bp NsiI-EcoRI fragment from pMON13418 | 76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S | Polypeptide C-9 SEQ ID NO:327 |
| Example 134 | pMON13459 SEQ ID NO:395 | pMON13428 NcoI-NsiI | 170 bp NcoI-NsiI fragment from pMON13402 | 23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T 109L 112Q 116S | Polypeptide C-12 SEQ ID NO:328 |
| Example 135 | pMON13467 SEQ ID NO:396 | pMON13413 NcoI-NsiI | 170 bp NcoI-NsiI fragment from pMON13402 | 23L 29I 34S 37S 38A 45V 46S 109L 112Q 116S 109L 112Q 116S 117S | Polypeptide C-13 SEQ ID NO:329 |
| Example 136 | pMON13492 SEQ ID NO:397 | pMON13418 NcoI-NsiI | 170 bp NcoI-NsiI fragment from pMON13402 | 23L 29I 34S 37S 38A 45V 46S 76P 79S 85V 87Y 91P 95T 98T | Polypeptide C-14 SEQ ID NO:330 |